A STUDY OF THE CAROTINOID PIGMENTS OF WHEAT AND FLOUR WITH SPECIAL REFERENCE TO WHEAT VARIETIES

By
A. G. O. WHITESIDE, B.S.A., M.S.

CEREAL DIVISION
DOMINION EXPERIMENTAL FARM

L. H. NEWMAN, B.S.A.
DOMINION CEREALIST

DOMINION OF CANADA
DEPARTMENT OF AGRICULTURE
BULLETIN No. 154—NEW SERIES

Published by Direction of the Hon. Robert Weir, Minister of Agriculture,
Ottawa, 1931

DOMINION EXPERIMENTAL FARMS BRANCH

PERSONNEL

DIRECTOR, E. S. ARCHIBALD, B.A., B.S.A., LL.D., D.Sc.

Dominion Field Husbandman.....E. S. Hopkins, B.S.A., M.S.
Dominion Chemist.....Frank T. Shutt, M.A., D.Sc.
Dominion Horticulturist.....W. T. Macoun, D.Sc.
Dominion Cerealist.....L. H. Newman, B.S.A.
Dominion Botanist.....H. T. Güssow.
Dominion Animal Husbandman.....
Dominion Agrostologist.....
Dominion Poultry Husbandman.....F. C. Elford.
Chief, Tobacco Division.....N. T. Nelson, B.S.A., M.S., Ph.D.
Dominion Bacteriologist.....Grant Lochhead, B.A., M.Sc., Ph.D.
Dominion Apiarist.....C. B. Gooderham, B.S.A.
Chief, Division of Extension and Publicity.....F. C. Nunnick, B.S.A.
Chief Supervisor of Illustration Stations.....J. C. Moynan, B.S.A.
Economic Fibre Specialist.....R. J. Hutchinson.

ALBERTA

Superintendent, Experimental Station, Lacombe, Alta., F. H. Reed, B.S.A.
Superintendent, Experimental Station, Lethbridge, Alta., W. H. Fairfield, M.Sc., LL.D.
Superintendent, Experimental Sub-station, Beaverlodge, Alta., W. D. Albright.
Superintendent, Experimental Sub-station, Fort Vermilion, Alta., Robt. Jones.

BRITISH COLUMBIA

Superintendent, Experimental Farm, Agassiz, B.C., W. H. Hicks, B.S.A.
Superintendent, Experimental Station, Summerland, B.C., W. T. Hunter, B.S.A.
Superintendent, Experimental Station, Windermere, B.C., R. G. Newton, B.S.A.
Superintendent, Experimental Station, Sidney, B.C., E. M. Straight, B.S.A.

MANITOBA

Superintendent, Experimental Farm, Brandon, Man., M. J. Tinline, B.S.A.
Superintendent, Experimental Station, Morden, Man., W. R. Leslie, B.S.A.

SASKATCHEWAN

Superintendent, Experimental Farm, Indian Head, Sask., W. H. Gibson, B.S.A.
Superintendent, Experimental Station, Rosthern, Sask., W. A. Munro, B.A., B.S.A.
Superintendent, Experimental Station, Scott, Sask., G. D. Matthews, B.S.A.
Superintendent, Experimental Station, Swift Current, Sask., J. G. Taggart, B.S.A.

NEW BRUNSWICK

Superintendent, Experimental Station, Fredericton, N.B., C. F. Bailey, B.S.A.

NOVA SCOTIA

Superintendent, Experimental Farm, Nappan, N.S., W. W. Baird, B.S.A.
Superintendent, Experimental Station, Kentville, N.S., W. S. Blair, D.Sc.

PRINCE EDWARD ISLAND

Superintendent, Experimental Station, Charlottetown, P.E.I., J. A. Clark, M.S.A., D.Sc.

ONTARIO

Central Experimental Farm, Ottawa, Ont.
Superintendent, Experimental Station, Kapuskasing, Ont., S. Ballantyne.
Superintendent, Experimental Station, Harrow, Ont., H. F. Murwin, B.S.A.

QUEBEC

Superintendent, Experimental Station, Cap Rouge, Que., G. A. Langelier, D.Sc.A.
Superintendent, Experimental Station, Lennoxville, Que., J. A. McClary.
Superintendent, Experimental Station, Ste. Anne de la Pocatière, Que., J. A. Ste. Marie, B.S.A.
Superintendent, Experimental Station, La Ferme, Que., P. Fortier, Agr.
Superintendent, Experimental Station, Farnham, Que., R. Bordeleau, B.S.A.
Superintendent, Experimental Station, L'Assomption, Que., J. E. Montreuil, B.S.A.

TABLE OF CONTENTS

DOMINION OF CANADA
DEPARTMENT OF AGRICULTURE

BULLETIN NO. 154—NEW SERIES

29469—1

A Study of the Carotinoid Pigments of Wheat and Flour with Special Reference to Wheat Varieties

BY

A. G. O. Whiteside, B.S.A., M.S.

INTRODUCTION

The growing of wheat for human consumption is one of the oldest of agricultural practices. As civilization progressed improvement was manifested in better varieties and in better methods in the milling of wheat. It is not the purpose to review the history of wheat and wheat milling improvement but to point out that there was a gradual evolution in these two closely connected fields which kept pace with the advancement of civilization. With the revolution brought about by the new discoveries of the machine age, and the development of science in industry and agriculture, rapid strides have been made in the growing of wheat and the milling of wheat into flour. As the knowledge grew concerning the mode of reproduction in plants, certain biological principles were discovered which led to the development of the science of plant breeding. The application of genetics to the breeding of wheat now makes it possible to undertake definite programs to effect an intelligent improvement in the characters of certain wheat varieties.

Likewise the milling industry has shown great progress. The advent of the automatic steel roller system, or sometimes referred to as the Hungarian System, the middlings purifier and improved methods of conditioning wheat for the rolls have had the effect of making a sharper separation of the wheat endosperm from the offal and the production of more refined products. These mechanical developments, together with the more recent artificial bleaching of flour have made possible the production of whiter flour, and have resulted in a demand from the public for the whiter product due possibly to the association of whiteness with purity and cleanliness.

The preference of the public for flour of light colour was early recognized by plant breeders as numerous reports in the milling and baking test of wheat varieties included a system of scoring whereby those wheats which baked into loaves with light coloured crumbs were given higher ratings in this respect than those which produced crumbs more yellow or dark. Varieties differ in the quantity of yellow pigments contained in their kernels and the flour milled from them. Those which are abnormally yellow are usually viewed with suspicion by the milling trade, even though many mills are equipped to bleach flour. Then, again, the bleaching of flour is not looked upon very favourably by some countries or districts of certain countries. The milling trade is a keen competitive business and it would appear that it prefers wheats which are naturally low in yellow pigments.

Cereal chemists are aware of the recent evidence of Moore (1930) and Capper (1930) and others supporting the view that carotin, the chief yellow pigment of wheat flour, may be the provitamine of Vitamine A. It is now believed that carotin when taken into the animal body is changed in some manner to give Vitamine A activity. If it should happen that the bleaching of flour is not desirable from the standpoint of destroying Vitamine A, it would seem that wheats which are naturally low in carotinoid pigments would mill into flours with a fair amount of the provitamine and produce loaves of fairly light crumb colour. However, wheat is not considered a plentiful source of Vitamine A and any arguments against the bleaching of flour from this angle would not be strongly supported.

CAROTINOID PIGMENTS IN WHEAT AND FLOUR

The naturally occurring carotinoid pigments in wheat are responsible for the yellow colouring matter in flour. Carotinoid is the term used to classify those pigments which are usually red, orange or yellow, and which can be extracted by the use of fat solvents from biological tissues either of plant or animal origin. Monier-Williams (1912) compared the absorption spectra of petroleum ether extracts of flour with similar solutions of carotin and came to the conclusion that the colouring matters are either identical or closely related to carotin, the position of the bands in both cases being practically the same. Ferrari and Bailey (1929) confirmed the work of Monier-Williams by comparing the absorption spectra of gasoline extracts of flour with the absorption spectra of pure carotin in petroleum ether. They state that the absorption spectra secured from the extract of flour does not constitute proof that the pigment present was carotin alone, but conclude that the pigment of flour is essentially carotin. Ferrari and Bailey noted that the slight shifting of the bands to the left, observed in the spectral plates, may be explained on the difference in the density of the gasoline with that of petroleum ether, even though the characteristic bands of xanthophyll are to the left of those of carotin.

Palmer (1922) noted the presence of carotin and xanthophyll in the wheat (Triticum vulgare). Ferrari and Bailey (1929) compared the absorption spectra of the gasoline extracts from washed bran with that from the flour and with pure carotin in petroleum ether and found no similarity whatever to the absorption spectra of either flour extract or pure carotin. They described the hue of the gasoline extract of the bran as decidedly reddish in colour. Carotin, then, is considered the chief carotinoid pigment in wheat and flour. There is some evidence that xanthophyll and an undetermined pigment which imparts a reddish hue to gasoline extracts of bran are also present in the wheat kernel.

CAROTIN AND ITS PROPERTIES

From the foregoing it is fairly well established that the chief pigment responsible for the yellow colour of wheat flour is carotin. It is an unsaturated hydrocarbon with the formula $C_{40}H_{56}$. The structural formula has not yet been determined, although partial structures have been proposed (Gullard 1930). Carotin crystals are readily oxidized and bleach entirely. Palmer (1922) reviewing the literature cites that the amount of oxygen which carotin can take up has been reported by Arnaud to be 21 to 24 per cent; by Kohl to be as high as 37 per cent; by Willstätter and Mieg to be 34·3 per cent, and by Willstätter and Escher to be the amount required to form an oxidized product corresponding to nearly 12 atoms of oxygen.

Carotin forms addition products with iodine, two of which have been described, $C_{40}H_{56}I_2$ and $C_{40}H_{56}I_3$. Bromine both adds and substitutes to form a stable compound $C_{40}H_{36}Br_{22}$.

Carotin crystallizes from carbon bisulphide on the addition of absolute alcohol. It crystallizes from petroleum ether in quadratic plates. Carotin in dilute solutions with petroleum ether is yellow but in concentrated solutions it is ruby red.

Schertz (1925) determined the solubilities of carotin in the following solutions at 25° C.: absolute alcohol 15.5 mgms. per litre; petroleum ether (B.P. 30°-50° C.) 626 mgms. per litre, ethyl ether 1005 mgms. per litre. Solutions of carotin in absolute alcohol and petroleum ether were extremely stable when stored in the ice-box but in other solutions decomposed rapidly. Carotin is slightly soluble in acetone and readily soluble in chloroform. Carotin in the presence of lipoids will dissolve in 95 per cent alcohol. Solutions of carotin are unaffected by boiling with alkalis and may be recovered unchanged from such solutions. Carotin is adsorbed from petroleum ether solution by finely divided Hg Cl_2, $CaCl_2$ and PbS. It is not adsorbed by calcium carbonate,

inulin or powdered sucrose. It is distinguished from xanthophyll in that the latter is adsorbed by calcium carbonate; also carotin can be quantitatively removed from 80 to 90 per cent alcohol by shaking with carbon bisulphide or petroleum ether and cannot be removed from petroleum ether when shaken with 80 to 90 per cent alcohol.

Solutions of carotin in alcohol and the fat solvents exhibit characteristic absorption spectra. Willstätter and Stoll (1913) determined the absorption spectrum of pure carotin in alcohol at a concentration of 5 mgms. per litre in a 5 mm. and 10 mm. cell.

	5 m.m. cell	10 m.m. cell
Band I	492-478 m μ	492-476 m μ
Band II	459-446 m μ	459-445 m μ
End absorption	415-	419-

Ferrari and Bailey (1929) obtained the absorption spectrum of pure carotin in petroleum ether in a 10 cm. cell at a concentration of 0·68 mg. per litre.

	10 cm. cell
Band I	493-477 m μ
Band II	460-442 m μ
Band III	425 m μ

THE QUANTITATIVE MEASUREMENT OF CAROTINOIDS

Advantage has been taken of the solubility of the carotinoids in fat solvents for their quantitative measurement. Thus Winton (1911) devised the gasoline colour value of flour which was adopted by the Association of Official Agricultural chemists as a tentative method. The extract obtained is compared with an 0·005 per cent aqueous solution of potassium chromate in a colorimeter. Willstätter and Stoll (1913) working with carotin and xanthophyll used a solution prepared from 2 grams of potassium dichromate dissolved in 1 litre of distilled water as a substitute comparison solution for a known quantity of carotin dissolved in petroleum ether and xanthophyll dissolved in ether. Schertz (1923) working with pure carotin compared the spectrophotometer with the colorimeter as to accuracy in determining the carotin content of solutions. The spectrophotometer gave excellent results which was not possible with the colorimeter owing to the difficulty in matching solutions. Numerous attempts have been made to improve the colorimetric methods by improving the standard comparator solutions and by modifications in colorimeters. Jørgensen (1927) indicated that solutions of chromates and dichromates must be considered hydrogen ion indicators and that by proper use of buffer solutions more reliable colour standards may be produced. Kent-Jones and Herd (1927) suggested a standard solution for comparing gasoline extracts of flour made up of a solution of potassium chromate and cobalt nitrate. Kent-Jones and Herd (1927) devised a special colorimeter which is independent of daylight. Visser't Hooft and De Leeuw (1928) compared the Kent-Jones and Herd colorimeter with a Duboscq colorimeter and found a great difference between the values obtained with the two colorimeters.

It will be inferred from the above discussion that colorimetric methods for measuring the concentration of carotinoid pigments are open to error. Schertz (1923) devised an accurate method for the measurement of pure carotin through the use of the spectrophotometer. He states that the transmittancies as measured depend upon the physical properties of the substance involved and not upon variability in light, physiological factors, or tint of solution, which so greatly affect the readings in any colorimeter, and are independent of abnormalities of the observer's colour vision. Schertz determined the specific trans-

missive index for dilute solutions of carotin in petroleum ether. Knowing this value it is not necessary to prepare pure carotin to be used as a standard or to prepare any other standard as the spectrophotometer itself is standardized in regard to carotin when the transmittancy of the solution is known. Ferrari and Bailey (1929) adapted the method of Schertz for the quantitative determination of solutions of pure carotin in petroleum ether to the quantatitive determination of carotin in the gasoline extracts of flour and proposed a new conventional method for the determination of the carotin in flour. This method formed the basis for the investigations reported in this paper.

THE APPLICATION OF THE SPECTROPHOTOMETER TO THE DETERMINATION OF CAROTIN

The principles underlying the application of the spectrophotometer have been ably described by Schertz (1923) and by Ferrari and Bailey (1929) but it is thought that a brief review would not be amiss here. By the use of the spectrophotometer it is possible to measure the relative amount of light transmitted in any wave length by a cell containing a pigmented solution. Solutions of carotin have the greatest absorption in the blue to violet end of the spectrum and it is therefore desirable to make measurements in this range as small differences in concentration will be readily reflected in the relative absorption of light passing through the solution. Schertz (1923) adopted the mercury line 435·8 m μ as the most satisfactory wave length for obtaining accurate results with carotin solutions. The transmission or transmittance of the light for this wave length is the ratio of the light passing through the last surface of cell to light incident on first surface of cell. A five centimeter cell made of optical glass with a partition in the centre dividing it into two parts, one side for the solvent and the other for the solution, was used in connection with the spectrophotometer in obtaining subsequent data. Since the glass surfaces are identical for the solvent and solution, the transmission refers to the liquid in the cell only.

The transmission of the solution may be designated as (Sol T) while the transmission of the solvent in the duplicate cell (Sov T). Then transmittancy (T) which may be defined as the ratio of the transmission of a cell containing the solution to the transmission of a duplicate cell containing the solvent becomes

$$T = \frac{(\text{Sol T})}{(\text{Sov T})} = \frac{\text{Sol T}}{\text{Sov T}}$$

In the above relation it will be seen that the transmittancy will be corrected for any absorption of light by the solvent.

Transmissivity may be defined as equal to the "b" root of the transmittance where "b" is the length of the layer of liquid in centimetres.
It is expressed:

$t = \sqrt[b]{T}$ which is the relation known as Lambert's law.

The Specific Transmissivity $= \sqrt[bc]{T}$

where c = concentration of its dissolved substance in centigrams per litre.
The Specific Transmissive Index (the extinction coefficient or absorption index)

$$= K = -\log_{10} t = -\frac{1}{bc} \log_{10} T$$

$$bcK = -\log_{10} T$$

This relation is known as Beer's Law.

Schertz (1923) has shown that for dilute solutions of carotin Beer's law holds. This can be stated that the transmissive index K is constant regardless of thickness or concentration. Schertz (1923) determined the value K for

dilute solutions of carotin to be 1·9148, while Ferrari and Bailey (1929) obtained the value 1·9156. These are in very good agreement with each other.

Ferrari and Bailey (1929) show a graph which can be used in the quantitative estimation of carotin. The data calculated from the values obtained by Schertz (1923) on pure carotin of known concentration in petroleum ether are plotted in the following manner:

"The concentration of carotin is plotted on an equal division scale along the axis of abscissa. The percentage transmittancy is plotted on the logarithmic scale along the left-hand ordinate, while the right hand ordinate indicates the corresponding values for the negative logarithm of the transmittancy".

The straight line relation for three cells, 1 cm., 2 cm. and 10 cm., between the concentration of carotin and the logarithm of the percentage tramsmittancy are shown. From the formula $bck = -\log_{10}T$ knowing the length of the cell b, the constant k and determining the transmittancy (T) of the carotin solution, the concentration of carotin may be obtained directly from the graph or by calculation.

COLOUR DETERMINATIONS OF WHEAT AND FLOUR

Winton (1911) reports the gasoline colour values of patent and clear flours from four typical kinds of wheat. The values obtained, together with some of the chemical data on the new or freshly milled flour taken from Winton's data are reported in the following Table I:

TABLE I—GASOLINE COLOUR VALUES OF FRESHLY MILLED, PATENT AND CLEAR FLOURS, PRODUCED FROM FOUR TYPES OF WHEAT

Description of samples	Minnesota hard spring		Nebraska hard winter		Michigan soft winter		Missouri soft winter	
	78 % patent	22 % clear	80 % patent	20 % clear	80 % patent	20 % clear	40 % patent	60 % clear
Gasoline colour								
Value	2·00	2·00	2·63	2·50	1·43	1·61	1·47	1·60
Ash	0·44	0·85	0·39	0·67	0·42	0·89	0·39	0·50
Crude fibre	0·06	0·26	0·18	0·24	0·19	0·27	0·34	0·38
Protein (N x 5·7)	10·60	11·74	10·09	11·86	8·66	12·26	9·01	10·72
Fat	1·09	1·98	0·85	1·32	1·11	1·77	0·87	1·15
Moisture	13·74	13·26	13·33	12·85	13·22	12·62	12·27	12·02

It will be noted that the gasoline colour values of the patents and clears do not appear to differ appreciably. Furthermore there does not seem to be any quantitative relation between the protein, ash, fibre and fat content of the flour and gasoline colour values.

Coleman and Christie (1926) studied the gasoline colour values of straight grade flours of several classes of wheat and obtained considerable variation among the samples of each class. The largest value 2·40, was obtained from a sample of hard white wheat and the lowest, 0·64, from a sample of soft red winter wheat. Table II is taken from Coleman and Christie's data.

TABLE II—GASOLINE COLOUR VALUES OF WHEAT FLOUR MILLED FROM DIFFERENT WHEAT CLASSES

—	Hard red spring	Hard red winter	Soft red winter	Hard white	Common white
Number of samples	18	22	10	8	5
Average	1·39*	1·69	1·67	1·41	1·13
Maximum	1·55	2·06	1·87	1·95	1·22
Minimum	1·25	1·44	1·60	1·32	1·00
Range	0·30	0·62	0·27	0·63	0·22

* The value 1 is equal in colour intensity to 0·005 per cent potassium chromate aqueous solution.

Correlation studies made between the gasoline colour values of 194 durum wheat flours and protein content and ash content revealed no relationship in either case.

In addition Coleman and Christie (1926) reported that good results had been obtained in determining gasoline colour values on ground wheat when the sample was finely ground until at least 75 per cent of it passed through a No. 50 grit gauze sieve.

Ferrari and Bailey (1929) reported on the concentration of carotin in the flour streams which go into the straight grade flour of the Minnesota State Testing Mill. Their values indicated that the yellow colouring matter was well distributed in the various fractions. The first break flour contained the lowest and the fifth break flour the highest. The fourth break flour and the bran, shorts and duster flour contained high concentrations of carotin but these constituted a relatively small proportion of the flour making up the straight grade product. Transmittancy measurements made on the various products from the same sample of wheat were obtained and the values translated into carotin in parts per million.

Bran	2·88	parts per million
Shorts	3·40	"
Red dog	2·85	"
Straight grade flour	2·43	"

Ferrari and Bailey (1929) determined the efficiency with which gasoline extracts carotin from milled products. A sample of Marquillo wheat was divided into four parts—patent flour, clear flour, shorts and bran. The sum total of the carotin in parts per million of these products proportioned in the ratio of per cent product was in substantial agreement with that obtained on the whole wheat finely ground. A composite of the products representing the whole wheat was prepared. The values determined in this were in agreement with the other values. Further, an experiment was conducted in order to ascertain if the bran was a factor influencing the amount of pigments that could be extracted from a sample containing flour and bran by gasoline. Separate determinations were made on the extracts of flour, and of finely ground bran, and of these products when mixed in various proportions. No indication was found that the bran limited the quantity of pigments extracted from the mixtures. Ferrari and Bailey state that had a substantial amount of carotin been adsorbed by the bran this fact should have been apparent.

EXPERIMENTAL

PART I

The Problem.—The study resolved itself into a comparison of the carotin content of laboratory-milled flour from twenty-two varieties of Spring wheat as determined by the Ferrari and Bailey method (1929) and the relation of these results to other characteristic data on their wheats, flours and test loaves.

The Material.—Composite samples of the wheat varieties grown in replicated rod row plots on the Dominion Experimental Farm, Lethbridge, Alberta, in 1929, were used in this experiment. One series of twenty-two samples was grown on land which was designated as dry land, and a second series of the same varieties was grown on land designated as irrigated land. Samples derived from replicated rod row plots are particularly desirable as the error due to soil variation is usually greatly reduced. The dry land series was grown on sandy clay loam in a rotation of summerfallow and grain. The irrigated land series was grown on sandy clay loam in a rotation of corn, peas and grain. Both of these areas have been in these rotations for many years. The

crops on the dry land area have never received any irrigation and the crops on the irrigated area have been supplied water each season according to the demands of the crop. Occasionally, as in 1928, no irrigation was given on account of the unusual precipitation throughout the season. In 1929 the wheat land was irrigated shortly after seeding but no further water was supplied during the remainder of the year.

The Varieties under Investigation were as follows:—

Hard Red Spring—Early Red Fife, Early Triumph, Garnet, Huron, Kitchener, Marquis, Ceres, Reward, Red Fife, Red Bobs No. 222, Renfrew, Ruby, Supreme, Marquis 10 B, Fisher's 1 B, Fisher's 2 B, and 928 QQ2.

White Spring—Hard Federation 71, White Federation 4981, Hard Federation 31, Hard Federation 4733, Quality.

All of these samples were sound, well matured and free from other grains and foreign material.

The flour represented all the good flour which could be milled out of each sample.

METHODS

Experimental Milling—An Allis-Chalmers experimental mill with two breaks and three reduction units was used for milling the wheat.

Moisture tests were made on each sample and sufficient grain was weighed out in each case to make two thousand grammes on a 13·5 per cent moisture basis. The average moisture content of the samples in the dry land series was 8·6 per cent and the irrigated series was 8·8 per cent. The conditioning period was conducted in two stages. Sufficient water was added to each sample to bring the moisture content up to 13 per cent and the samples were stored in air-tight containers at room temperature for seven days to insure penetration of moisture. One hour before milling 47 c.c. of warm water (32° C.) were added to each sample to condition it to 15 per cent moisture.

An aliquot of the flour from each milling was stored in an air-tight bottle. It was not convenient to hold the flour at ice-box temperature until a few days had elapsed after milling, but from then until the flour-colour readings were taken the samples were stored at $\pm$ 4° C. All readings were completed within a month after the samples were milled.

The Method of Obtaining Gasoline Extracts of Flour.—The method of preparing samples was essentially the same as that set forth by Ferrari and Bailey (1929) which was as follows:—20 grammes of flour are weighed into a glass stopped bottle and 100 ml. of high test gasoline (colourless) are added. After thorough agitation the bottle is placed in a dark drawer or cupboard over night. The supernatant liquid is then decanted into a centrifuge tube. The tube is covered with rubber dam and whirled at high speed in a centrifuge for twenty minutes. The supernatant liquid is then siphoned off, using a special siphon tube. The latter is prepared by drawing out both ends of the glass siphon tube to a capillary and the end that is inserted into the liquid is bent back upon itself in the form of a letter "U". Every possible precaution is taken to siphon the liquid without disturbing the settled flour particles.

Colour Readings on Gasoline Extracts.—Readings were taken with a Schmidt and Haensch Spectrophotometer at a wave length of 435·8 m μ with a light source from a mercury vapour lamp. A five centimetre cell made of optical glass with a partition in the centre, one side for the solvent and the other side for the extract, was used. This cell was covered with a glass cover and mounted on the stage behind the prism for splitting the light. Five readings

were taken for each angle in the photometric field and the average of these obtained:—

Method of Calculating the Results.—The calculations on the quantitative estimation of carotin are based on Beer's Law which has been referred to elsewhere. A typical example may be given here.

b.c.k. = $-\log_{10}T$.
b. = thickness of cell in c.m.
c. = concentration in centigrammes per litre
k. = constant (specific transmissive index of carotin in petroleum ether solution)
T = transmission
$\theta 1$ = 38·2
$\theta 2$ = 58·1
T = cot $\theta 2$ tan. $\theta 1$
T = (cot 58·1) (tan. 38·2)
T = (0·62245) (0·78692)
T = 0·48981
(5) C. (1·916) = - log. 0·48981
C. = 0·309981

9·580

C. = 0·0324 centigrammes per litre
= 0·324 milligrammes per litre
C. in p.p.m. = 0·324 x 1,000,000 = 1·620

200,000

Test Weight per Bushel.—Grain grading equipment comprising a funnel with a tripod, an imperial quart brass measure, a wooden stroke and a torsion balance with special weights and beam were employed in taking test weight per bushel measurements.

Weight per 1,000 kernels.—The average weight in grammes of 250 kernels in duplicate was used as a basis for calculating weight of 1,000 kernels.

Crude Protein in Wheat.—Crude protein in wheat was calculated by determining the nitrogen and multiplying by the factor 5·7. The results were brought to 13·5 per cent moisture basis of the wheat.

Ash Content in Flour.—Ash determinations were made by the Magnesium-Acetate-Alcohol Method (1930). The figures obtained were calculated to a moisture basis of 13·5 per cent.

Baking Tests.—The baking procedure was based essentially on the standard 100 gm. flour procedure of the American Association of Cereal Chemists, with the modifications described by Geddes (1929).

THE DATA

Carotin Concentration Values—Dry Land versus Irrigated Land Series

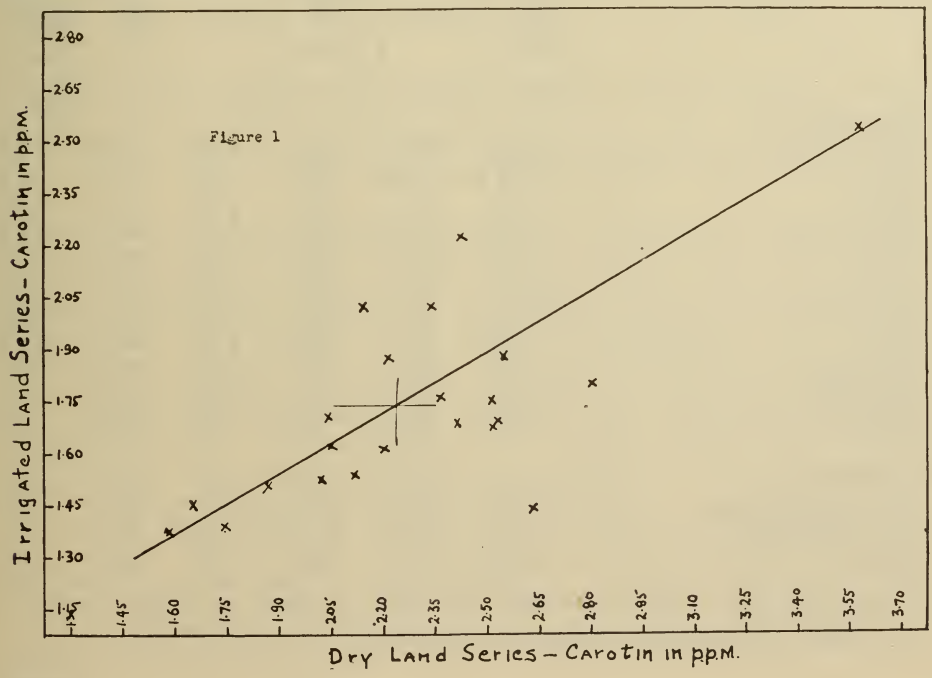
The gasoline colour values calculated as carotin in parts per million for each sample are recorded in Table III.

TABLE III—COMPARISON OF THE CAROTIN CONCENTRATION OF EXTRACTS OF FLOUR FROM DIFFERENT WHEAT VARIETIES GROWN ON DRY LAND AND ON IRRIGATED LAND

Variety	Carotin in p.p.m.*		
	Dry land series	Irrigated series	Dry-irrigated
Red Spring Wheats—			
Early Red Fife	2·42	2·22	0·20
Early Triumph	2·05	1·62	0·43
Garnet	2·21	1·88	0·33
Huron	2·41	1·80	0·61
Kitchener	2·34	2·02	0·32
Marquis	1·74	1·39	0·35
Ceres	2·53	1·69	0·84
Reward	1·59	1·38	0·21
Red Fife	2·20	1·61	0·59
Red Bobs 222	1·65	1·45	0·20
Renfrew	2·14	2·03	0·11
Ruby	2·02	1·53	0·19
Supreme	2·37	1·76	0·61
Marquis 10 B	2·63	1·44	1·19
Fisher's 1 B	3·58	2·54	1·04
Fisher's 2 B	2·52	1·68	0·84
928 Q.Q. 2	2·80	1·80	1·00
White Spring Wheats—			
Hard Federation 71	2·11	1·54	0·57
White Federation 4981	2·56	1·75	0·81
Hard Federation 31	2·04	1·71	0·33
Hard Federation 4733	2·55	1·88	0·67
Quality	1·87	1·52	0·35

* Parts per million.

In each case it will be observed that the values for the samples of the varieties grown on the dry land are higher than the values for the corresponding samples grown on the irrigated land. The differences in some cases are small,



but in others very appreciable. The averages for twenty-two samples grown on dry land and on irrigated land were 2·24 and 1·74 respectively. These indicate that flours milled from samples grown on the dry land carried a greater preponderance of yellow pigments than those milled from samples grown on the irrigated land. A simple product moment correlation, $r = 0{\cdot}7238 \pm 0{\cdot}0685$ was obtained between the figures for the varieties grown on dry land and the figures for the varieties grown on irrigated land. This is interpreted to mean that the carotin concentrations for the varieties grown on irrigated land followed the trend of the carotin concentrations for the varieties grown on dry land. In other words, there was a tendency for an inherent varietal influence contributing to the values obtained. This relation is better shown in figure 1.

The wide differences in carotin concentrations between the samples grown in the dry land series and those grown in the irrigated land series observed in some varieties make it desirable to proceed further in studying the samples in order to ascertain other factors which may have a bearing on the carotin concentrations obtained.

TEST WEIGHT PER BUSHEL* AND CAROTIN CONCENTRATION

Test weight per bushel measurements of the wheat samples and the carotin concentration values for the gasoline extracts from their corresponding flour samples are recorded in Table IV.

* The test weight per bushel measurements were determined in pounds avoirdupois per imperial bushel.

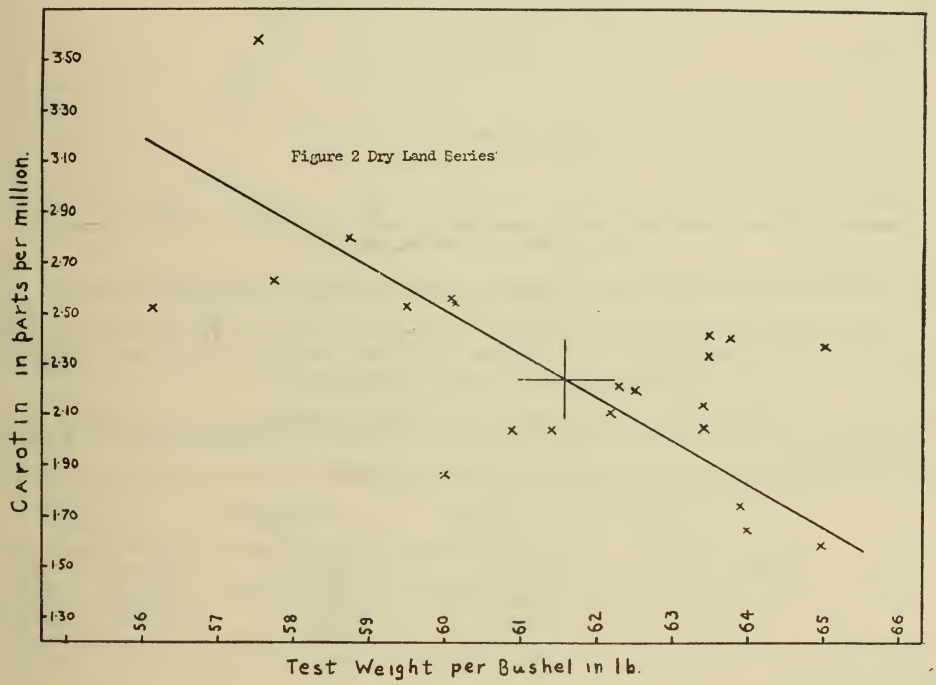
TABLE IV—A COMPARISON OF THE TEST WEIGHT PER BUSHEL OF THE WHEAT SAMPLES AND CAROTIN CONCENTRATION OF THEIR CORRESPONDING FLOUR SAMPLES

Variety	Dry land series		Irrigated land series		Dry minus irrigated	
	Test weight per bushel	Carotin	Test weight per bushel	Carotin	Test weight difference	Carotin concentration difference
	lb.**	p.p.m.*	lb.**	p.p.m.*	lb.**	p.p.m.*
Red Spring Wheats—						
Early Red Fife	63·5	2·42	64·1	2·22	−0·6	+0·20
Early Triumph	63·4	2·05	65·0	1·62	−1·6	+0·43
Garnet	62·3	2·21	64·2	1·88	−1·9	+0·33
Huron	63·7	2·41	64·5	1·80	−0·8	+0·61
Kitchener	63·5	2·34	64·2	2·02	−0·7	+0·32
Marquis	63·9	1·74	64·5	1·39	−0·6	+0·35
Ceres	59·5	2·53	65·2	1·69	−5·7	+0·84
Reward	64·9	1·59	66·1	1·38	−1·2	+0·21
Red Fife	62·5	2·20	62·8	1·61	−0·3	+0·59
Red Bobs 222	64·0	1·65	65·5	1·45	−1·5	+0·20
Renfrew	63·4	2·14	64·6	2·03	−1·2	+0·11
Ruby	61·4	2·02	64·1	1·53	−2·7	+0·19
Supreme	65·0	2·37	63·9	1·76	+1·1	+0·61
Marquis 10B	57·7	2·63	65·0	1·44	−7·3	+1·19
Fisher's 1B	57·5	3·58	65·3	2·54	−7·8	+1·04
Fisher's 2B	56·1	2·52	64·1	1·68	−8·0	+0·84
928 Q.Q. 2	58·7	2·80	64·7	1·80	−6·0	+1·00
White Spring Wheats—						
Hard Federation 71	62·2	2·11	66·1	1·54	−3·9	+0·57
White Federation 4981	60·1	2·56	65·2	1·75	−5·1	+0·81
Hard Federation 31	60·9	2·04	66·2	1·71	−5·3	+0·33
Hard Federation 4733	60·1	2·55	65·0	1·88	−4·9	+0·67
Quality	60·0	1·87	65·2	1·52	−5·2	+0·35

* Parts per million.
** To convert to U.S. equivalents multiply by the factor 0·97.

It will be observed that the values for test weight per bushel of the samples in the irrigated land series are relatively higher than those in the dry land series, and that greater variations between the samples were shown in the latter series. In general the varieties which show the greatest differences in test weight per bushel when comparing the two series, exhibit the widest spread in carotin concentration. Thus, the Marquis 10 B sample, in the irrigated land series, is 7·3 pounds higher in test weight and 1·19 parts per million lower in carotin concentration. This would indicate that there may be some relation between test weight and carotin concentration.

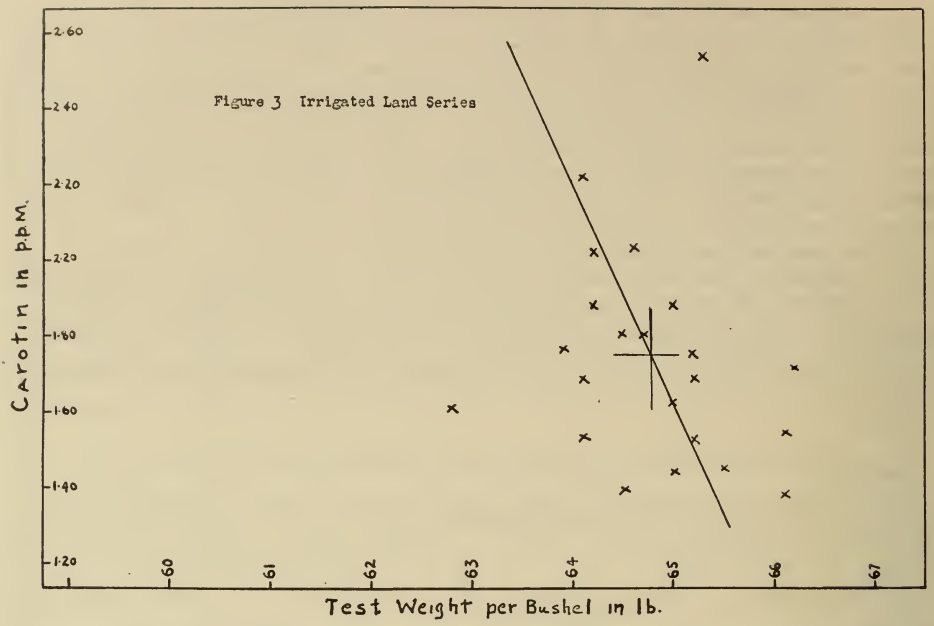
Disregarding the influence of variety in the calculation of simple correlation coefficients for each series, the values $r = -0{\cdot}6663 \pm 0{\cdot}0800$, and $r = -0{\cdot}1858 \pm 0{\cdot}1388$ were obtained for the dry land and irrigated land series, respectively. These relations are better shown in the correlation surfaces of



figures 2 and 3. Notwithstanding the effect of variety on the carotin concentrations there is some evidence in figure 2 that where the test weight is low the carotin concentration is likely to be high. In figure 3, owing to the lack of variation in test weight per bushel in the irrigated land series, these values are shown to have little bearing on the carotin concentrations. The correlation coefficient was calculated between test weight per bushel differences and carotin concentration differences, obtained by subtracting the values for each variety in the irrigated land series from the values for each corresponding variety in the dry land series. The correlation $r = -0{\cdot}6913 \pm 0{\cdot}0750$ was obtained which indicates that a decrease in carotin concentration was related to an increase in test weight per bushel in consequence of irrigation.

It would seem logical to conclude that the degree of development of the wheat kernels as indicated by test weight per bushel measurements exercises an influence on the carotin concentrations of the flours milled from them. Samples of the same variety which are shrunken and not fully developed are likely to produce flours with higher yellow pigment values than those which are plump and well developed.

29469—3½



WEIGHT PER THOUSAND KERNELS AND CAROTIN CONCENTRATION

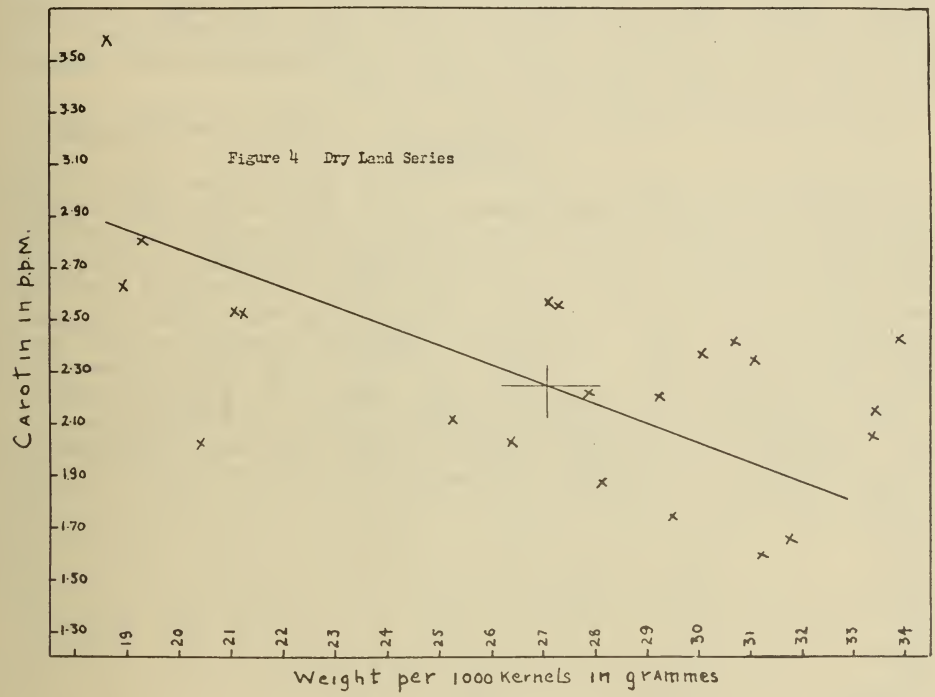
The weight per thousand kernels is a measure of the size, plumpness, and density of the wheat sample. It differs from test weight per bushel in that it is influenced to a greater extent by the size of the kernels.

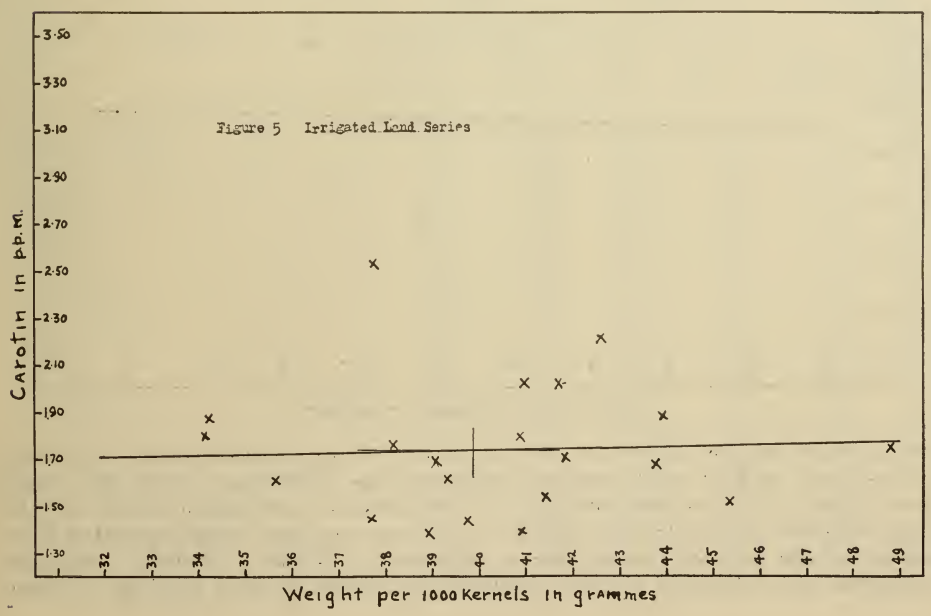
TABLE V—A COMPARISON OF THE WEIGHT OF 1,000 KERNELS OF THE WHEAT SAMPLES AND CAROTIN CONCENTRATION OF THEIR CORRESPONDING FLOUR SAMPLES

Variety	Dry land series		Irrigated land series		Dry minus irrigated	
	Weights per 1,000 kernels	Carotin	Weights per 1,000 kernels	Carotin	Weight per 1,000 kernels difference	Carotin concentration difference
	gms.	p.p.m.*	gms.	p.p.m.*	gms.	p.p.m.*
Red Spring Wheats—						
Early Red Fife	33·90	2·42	42·60	2·22	− 8·70	+0·20
Early Triumph	33·36	2·05	39·32	1·62	− 5·96	+0·43
Garnet	27·88	2·21	34·24	1·88	− 6·36	+0·33
Huron	30·70	2·41	40·86	1·80	−10·16	+0·61
Kitchener	31·04	2·34	41·66	2·02	−10·62	+0·32
Marquis	29·48	1·74	40·88	1·39	−11·40	+0·35
Ceres	21·08	2·53	39·06	1·69	−17·98	+0·84
Reward	31·18	1·59	38·92	1·38	− 7·74	+0·21
Red Fife	29·22	2·20	35·68	1·61	− 6·46	+0·59
Red Bobs 222	31·74	1·65	37·70	1·45	− 5·96	+0·20
Renfrew	33·44	2·14	40·98	2·03	− 7·54	+0·11
Ruby	20·40	2·02	31·00	1·53	−10·60	+0·19
Supreme	30·02	2·37	38·14	1·76	− 8·12	+0·61
Marquis 10B	18·92	2·63	39·74	1·44	−20·82	+1·19
Fisher's 1B	18·60	3·58	37·74	2·54	−19·14	+1·04
Fisher's 2B	21·20	2·52	43·72	1·68	−22·52	+0·84
928 Q.Q. 2	19·30	2·80	34·12	1·80	−14·82	+1·00
White Spring Wheats—						
Hard Federation 71	25·26	2·11	41·40	1·54	−16·14	+0·57
White Federation 4981	27·12	2·56	48·78	1·75	−21·66	+0·81
Hard Federation 31	26·36	2·04	41·82	1·71	−15·46	+0·33
Hard Federation 4733	27·30	2·55	43·94	1·88	−16·64	+0·67
Quality	28·22	1·87	45·38	1·52	−17·16	+0·35

* Parts per million.

In Table V it will be observed that the figures for weight per thousand kernels of the samples in the irrigated land series are higher than the corresponding samples in the dry land series. The wheat kernels in every sample of the former series were large and very plump. This was not the case in the latter series, as the kernels in some of the samples were small and only fair in plumpness. For example, Marquis 10 B, 928 Q.Q. 2, and Fisher's 1 B varieties were low in weight per thousand kernels in the dry land series but high in the irrigated land series. Simple correlation coefficients r = -0·5802 ± 0·0604 and



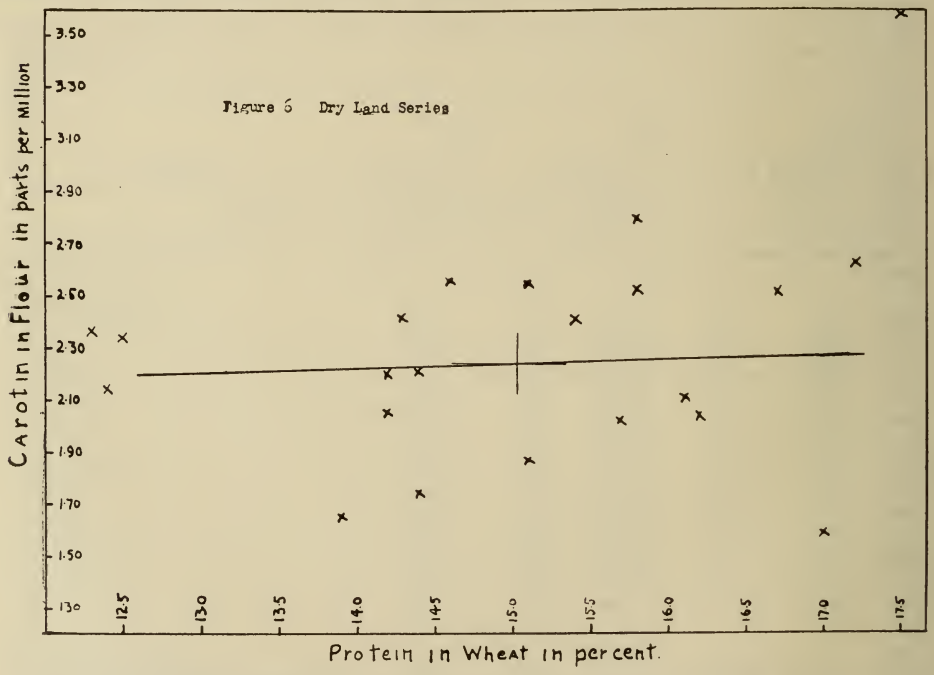


$r = 0 \cdot 0561 \pm 0 \cdot 1434$ between weights per thousand kernels of the wheat sample and carotin concentration values of the flours were obtained for the dry land and the irrigated land series, respectively. The correlation surfaces of figures 4 and 5 illustrate these relations.

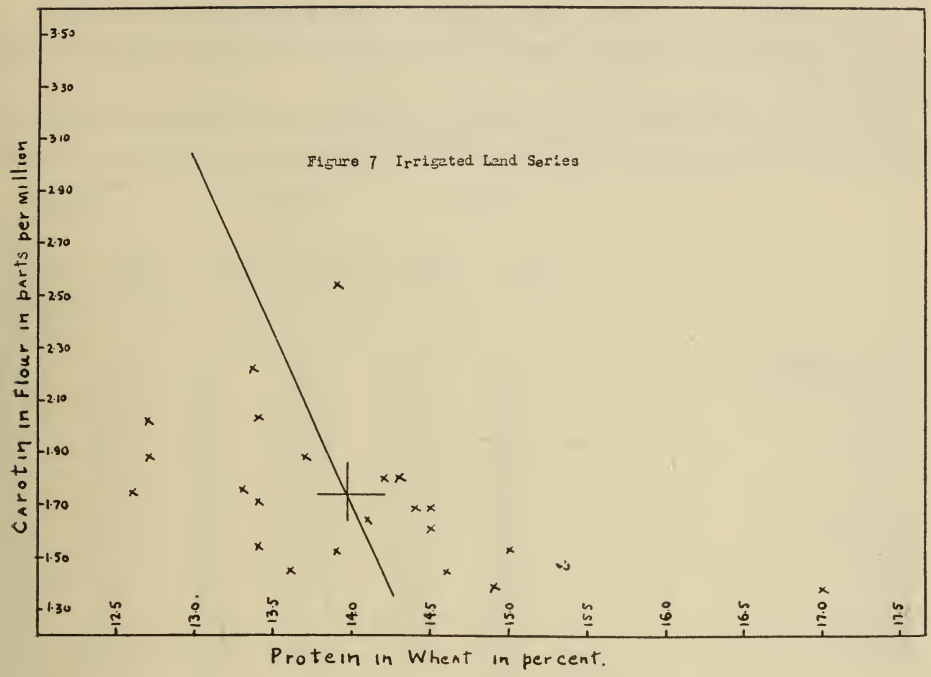
In addition the correlation coefficient was calculated between the weight per thousand kernel differences and carotin concentration differences, obtained in a similar manner to that between test weight per bushel differences and carotin concentration differences, and the value $r = -0 \cdot 7125 \pm 0 \cdot 0708$ was obtained. Since weight per thousand kernels and test weight per bushel measurements are influenced, primarily, by the degree of development of the wheat kernels it is not strange to observe that the simple correlation coefficients of the two sets of data are similar for both series.

CRUDE PROTEIN CONTENT OF WHEAT AND CAROTIN CONCENTRATION OF FLOUR

Comparisons are made in Table VI between crude protein content of the wheat samples and carotin concentration of the corresponding flour samples. The protein percentages of the wheat samples and the carotin concentrations of the flours averaged 15·03 per cent and 2·24 parts per million in the dry land series and 13·97 per cent and 1·74 parts per million in the irrigated land series. The question naturally arises, is protein content in the wheat related to carotin concentration in the flour? Correlation coefficients calculated on each series separately, gave the values $r = 0 \cdot 0305 \pm 0 \cdot 1437$ for the dry land series and $r = -0 \cdot 4955 \pm 0 \cdot 1086$ for the irrigated land series. The correlation surfaces for these comparisons are shown in figures 6 and 7. In addition, the correlation coefficient was calculated between protein percentage differences and



carotin concentration differences, obtained in the same manner as that described for the test weight and carotin concentration differences, and the value $r = -0 \cdot 1568 \pm 0 \cdot 1084$ was obtained. The lack of correlation shown in the dry land series was indicative that the protein percentage variations were independent of the carotin concentration variations. While a significant negative correlation was obtained in the irrigated land series it must be kept in mind



that varietal influence may have a bearing on this value, as it is easy to conceive that the varieties in this series which have a tendency to be higher in carotin concentration may have a tendency to produce lower protein. It is doubtful from the above evidence that protein content of the wheat is related to carotin concentration of the flour milled from it.

TABLE VI—A COMPARISON OF THE CRUDE PROTEIN CONTENT OF THE WHEAT SAMPLES AND THE CAROTIN CONCENTRATION OF THEIR CORRESPONDING FLOURS

Variety	Dry land series		Irrigated land series		Dry minus irrigated	
	Protein in wheat	Carotin in flour	Protein in wheat	Carotin in flour	Crude protein difference	Carotin concentration difference
Red Spring Wheats—	%	p.p.m.*	%	p.p.m.*	%	p.p.m.*
Early Red Fife	14·3	2·42	13·3	2·22	+1·0	+0·20
Early Triumph	14·2	2·05	14·1	1·62	+0·1	+0·43
Garnet	14·4	2·21	13·7	1·88	+0·7	+0·33
Huron	15·4	2·41	14·2	1·80	+1·2	+0·61
Kitchener	12·5	2·34	12·7	2·02	−0·2	+0·32
Marquis	14·3	1·74	14·9	1·39	−0·6	+0·35
Ceres	15·8	2·53	14·5	1·69	+1·3	+0·84
Reward	17·0	1·59	17·0	1·38	+0·0	+0·21
Red Fife	14·2	2·20	14·5	1·61	−0·3	+0·59
Red Bobs 222	13·9	1·65	13·6	1·45	+0·3	+0·20
Renfrew	12·4	2·14	13·4	2·03	−1·0	+0·11
Ruby	15·7	2·02	15·0	1·53	+0·7	+0·19
Supreme	12·3	2·37	13·3	1·76	−1·0	+0·61
Marquis 10B	17·2	2·63	14·6	1·44	+2·6	+1·19
Fisher's 1B	17·5	3·58	13·9	2·54	+3·6	+1·04
Fisher's 2B	16·7	2·52	14·4	1·68	+2·3	+0·84
928 Q.Q. 2	15·8	2·80	14·3	1·80	+1·5	+1·00
White Spring Wheats—						
Hard Federation 71	16·1	2·11	13·4	1·54	+2·7	+0·57
White Federation 4981	14·6	2·56	12·6	1·75	+2·0	+0·81
Hard Federation 31	16·2	2·04	13·4	1·71	+2·8	+0·33
Hard Federation 4733	15·1	2·55	12·7	1·88	+2·4	+0·67
Quality	15·1	1·87	13·9	1·52	+1·2	+0·35

* Parts per million.

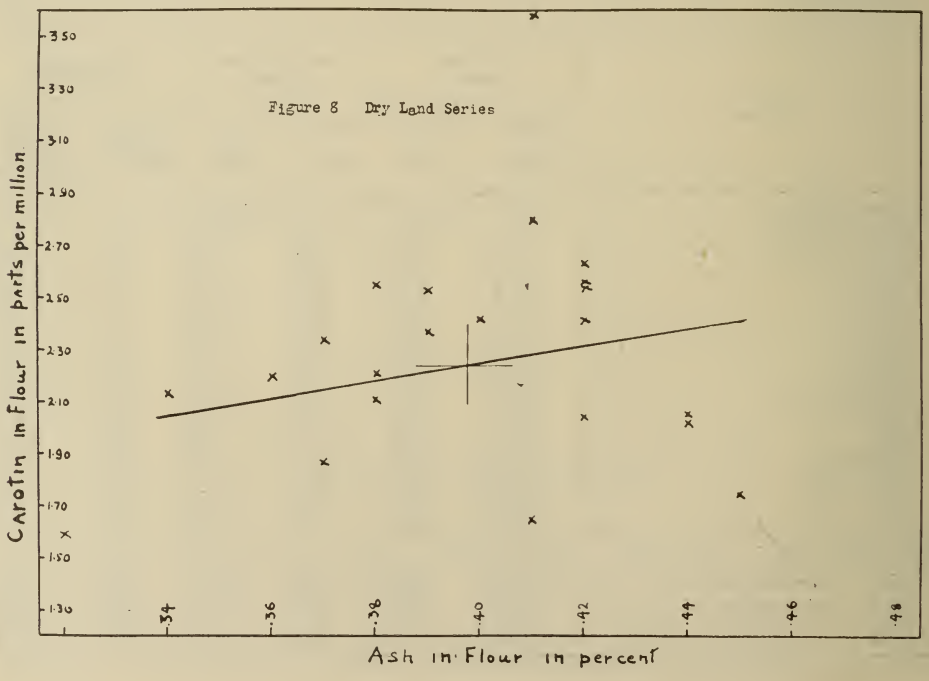
ASH CONTENT OF FLOUR AND CAROTIN CONCENTRATION OF FLOUR

The ash percentage and the carotin concentration for each flour are recorded in Table VII.

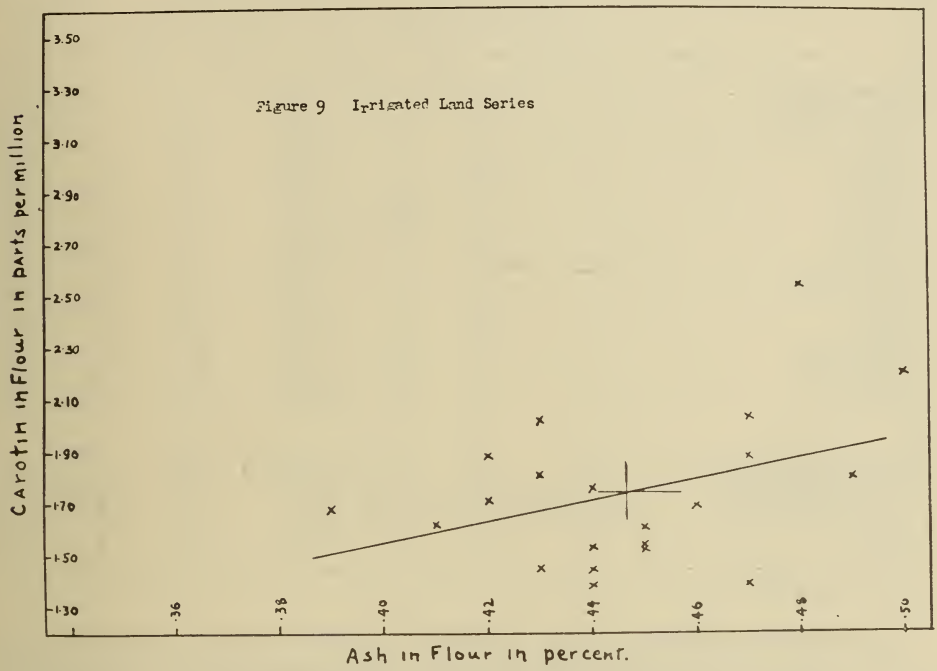
TABLE VII—A COMPARISON OF THE ASH CONTENT AND CAROTIN CONCENTRATIONS OF THE FLOURS

Variety	Dry land series		Irrigated land series		Dry minus irrigated	
	Ash in flour	Carotin in flour	Ash in flour	Carotin in flour	Ash content difference	Carotin concentration difference
	%	p.p.m.*	%	p.p.m.*	%	p.p.m.*
Red Spring Wheats—						
Early Red Fife	0·40	2·42	0·50	2·22	−0·10	0·20
Early Triumph	0·44	2·05	0·41	1·62	+0·03	0·43
Garnet	0·38	2·21	0·47	1·88	−0·09	0·33
Huron	0·42	2·41	0·49	1·80	−0·07	0·61
Kitchener	0·37	2·34	0·43	2·02	−0·06	0·32
Marquis	0·45	1·74	0·47	1·39	−0·02	0·35
Ceres	0·39	2·53	0·46	1·69	−0·07	0·84
Reward	0·33	1·59	0·44	1·38	−0·11	0·21
Red Fife	0·36	2·20	0·45	1·61	−0·09	0·59
Red Bobs 222	0·41	1·65	0·43	1·45	−0·02	0·20
Renfrew	0·34	2·14	0·47	2·03	−0·13	0·11
Ruby	0·44	2·02	0·44	1·53	0·00	0·19
Supreme	0·39	2·37	0·44	1·76	−0·05	0·61
Marquis 10B	0·42	2·63	0·44	1·44	−0·02	1·19
Fisher's 1B	0·41	3·58	0·48	2·54	−0·07	1·04
Fisher's 2B	0·42	2·52	0·39	1·68	+0·03	0·84
928 Q.Q. 2	0·41	2·80	0·43	1·80	−0·02	1·00
White Spring Wheats—						
Hard Federation 71	0·38	2·11	0·45	1·54	−0·07	0·57
White Federation 4981	0·42	2·56	0·45	1·75	−0·03	0·81
Hard Federation 31	0·42	2·04	0·42	1·71	0·00	0·33
Hard Federation 4733	0·38	2·55	0·42	1·88	−0·04	0·67
Quality	0·37	1·87	0·45	1·52	−0·08	0·35

* Parts per million.



It will be observed that the ash contents are in no case high for experimentally milled flour and indicate that the flour samples were comparatively free of branny particles and other parts of the kernel, usually considered as the offal or feed products. However, it was thought that a study of the relationship between the ash content and carotin content might be of interest and, therefore, correlation coefficients were calculated on these two sets of data.



The values $r = 0 \cdot 1642 \pm 0 \cdot 1399$ for the dry land series and $r = 0 \cdot 4110 \pm 0 \cdot 1414$ for the irrigated land series were obtained. In figures 8 and 9 the correlation surfaces are shown for these relations. The correlation coefficient between the ash content differences and the carotin concentration differences, obtained in a similar manner to that for test weight differences and carotin concentration differences, was found to be $r = -0 \cdot 1474 \pm 0 \cdot 1407$. Although a significant positive correlation was obtained in the irrigated land series it must be considered that varietal influence might have contributed to this correlation. The lack of correlation in the dry land series and in that obtained by a consideration of the differences, together with a consideration of varietal influence being a factor in the values for ash content and carotin content, there does not appear to be any justification to conclude that the carotin concentration values were related in any marked degree to the ash content in the flour.

DRY AND WET SLICK COLOUR COMPARISONS AND CAROTIN CONCENTRATIONS

The flour samples were compared for yellowness by smoothing them down on a strip of glass and estimating the relative yellowness for each sample.* The flours reflecting the greatest amount of yellow to the eye were given the highest ratings on a scoring basis from one to ten. In turn the slicked flours were immersed in water for twenty seconds and compared immediately following this operation. The colour comparisons this time were based on the colour of the film of paste formed by the imbibed water. In Table VIII a comparison is made between these values and carotin concentrations.

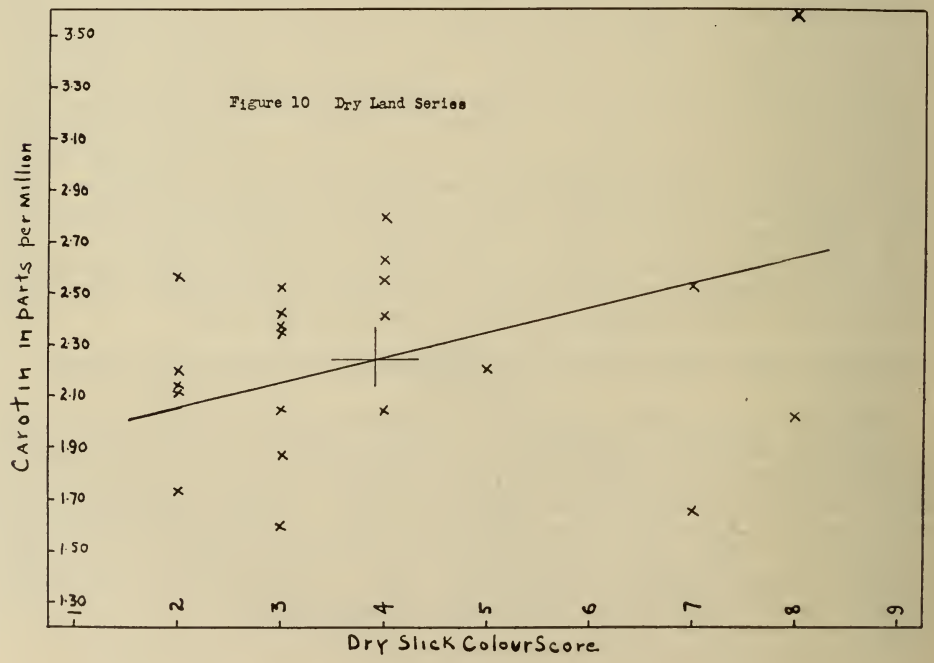
* The comparison of flours in this manner is usually termed "slicking" in the milling trade.

ABLE VIII—DRY AND WET SLICK COLOUR COMPARISONS AND CAROTIN CONCENTRATIONS OF FLOUR

Variety	Dry land series			Irrigated land series		
	Dry slick	Wet slick	Carotin	Dry slick	Wet slick	Carotin
	10–1	10–1	p.p.m.*	10–1	10–1	p.p.m.*
Red Spring Wheats—						
Early Red Fife	3	3	2·42	4	3	2·22
Early Triumph	3	2	2·05	2	3	1·62
Garnet	5	4	2·21	6	4	1·88
Huron	4	5	2·41	3	4	1·80
Kitchener	3	3	2·34	5	4	2·02
Marquis	2	2	1·74	2	4	1·39
Ceres	7	7	2·53	5	5	1·69
Reward	3	3	1·59	2	3	1·38
Red Fife	2	3	2·20	2	4	1·61
Red Bobs 222	7	4	1·65	5	4	1·45
Renfrew	2	3	2·14	3	2	2·03
Ruby	8	8	2·02	4	3	1·53
Supreme	3	3	2·37	3	4	1·76
Marquis 10B	4	5	2·63	8	7	1·44
Fisher's 1B	8	9	3·58	6	6	2·54
Fisher's 2 B	3	3	2·52	3	3	1·68
928 Q.Q. 2	4	4	2·80	4	4	1·80
White Spring Wheats—						
Hard Federation 71	2	5	2·11	1	6	1·54
White Federation 4981	4	4	2·56	4	5	1·75
Hard Federation 31	4	4	2·04	3	6	1·71
Hard Federation 4733	2	4	2·55	4	5	1·88
Quality	3	3	1·87	3	3	1·52

* Parts per million.

Correlation coefficients were calculated between carotin concentrations and dry and wet slick colour comparisons for the dry land and for the irrigated



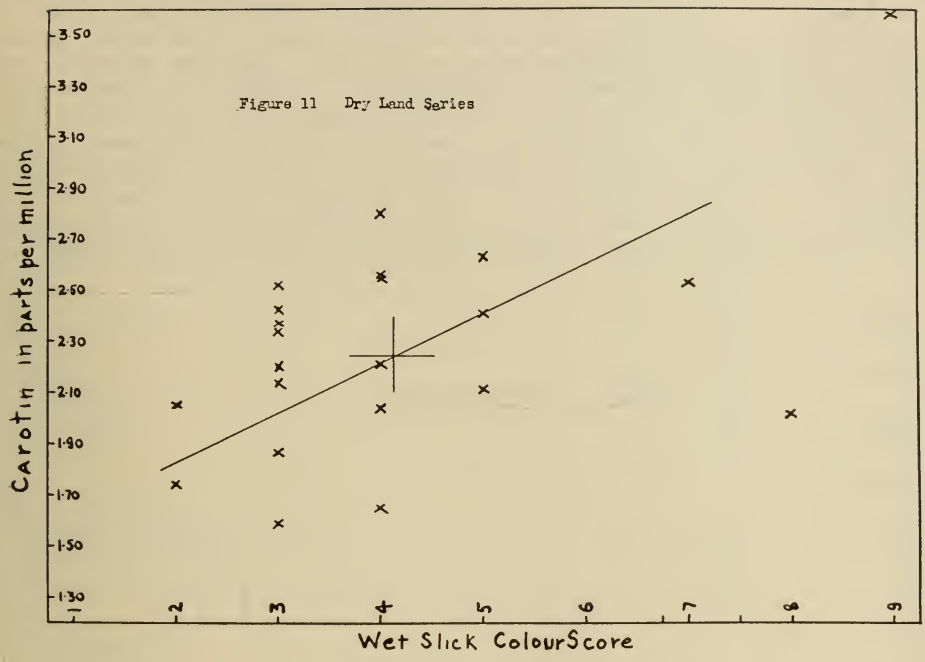


Figure 11 Dry Land Series
Carotin in parts per million
3·50
3·30
3·10
2·90
2·70
2·50
2·30
2·10
1·90
1·70
1·50
1·30
1
2
3
4
5
6
7
8
9
Wet Slick ColourScore

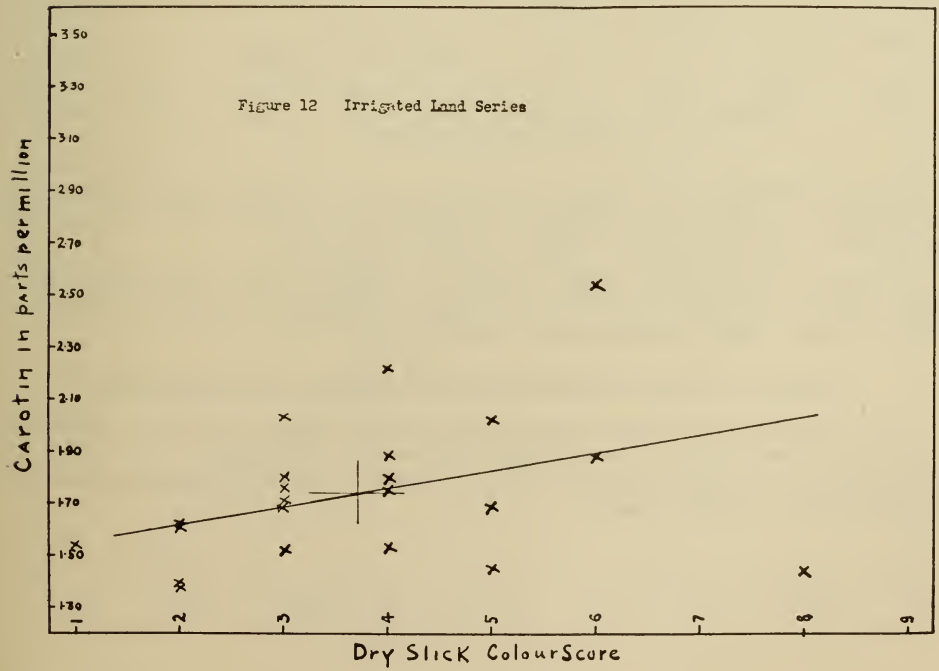
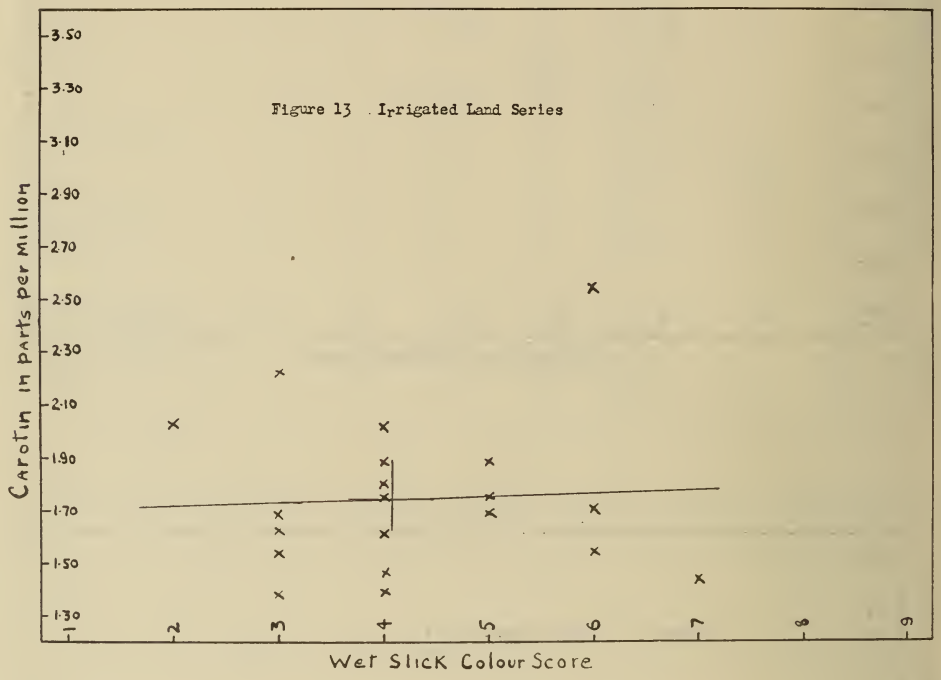


Figure 12 Irrigated Land Series
Carotin in parts per million
3·50
3·30
3·10
2·90
2·70
2·50
2·30
2·10
1·90
1·70
1·50
1·30
1
2
3
4
5
6
7
8
9
Dry Slick ColourScore

land series. For the dry land series the correlation coefficients r = 0·2896 ± 0·1317 and r = 0·5355 ± 0·1026 were obtained between carotin concentrations and the dry slick colour comparisons and between carotin concentrations and the wet slick colour comparisons, respectively. For the irrigated land series the correlation coefficients were found to be r = 0·3136 ± 0·1296 between carotin concentrations and the dry slick colour comparisons and r = 0·0467 ± 0·1435 between carotin concentrations and the wet slick colour comparisons. These relations are illustrated in figures 10, 11, 12 and 13. The correlation coefficients for the dry and wet slick colour comparisons and carotin concentrations in the dry land series and for the dry slick colour comparisons and carotin concentrations in the irrigated land series indicated a fairly significant



correlation between these colour estimations and carotin concentrations but no significant correlation was shown between the wet slick colour comparisons and carotin concentrations for the irrigated land series.

It must be concluded from the data presented that a very good agreement was not obtained between flour colour comparisons either in the dry or wet state and carotin concentration values of the flour.

CRUMB COLOUR SCORINGS AND CAROTIN CONCENTRATIONS

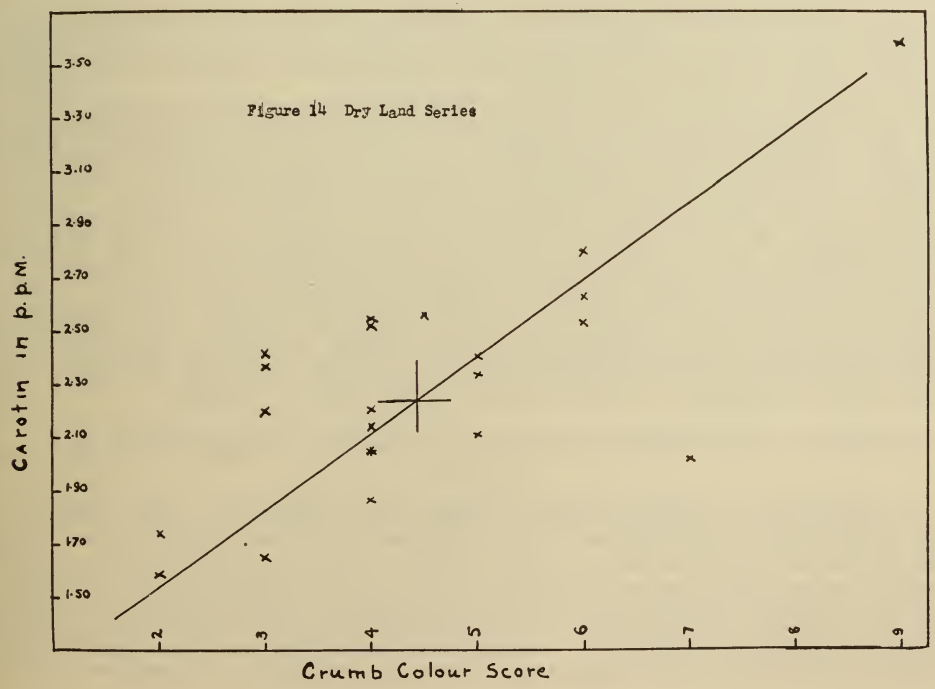
The crumbs of the baked loaves from the flours were scored for yellowness from one to ten, as in the case of the flour colour comparisons. In Table IX a comparison of these scorings with the carotin concentrations of the corresponding flour sample is made.

TABLE IX—CRUMB COLOUR COMPARISONS AND CAROTIN CONCENTRATIONS OF FLOUR

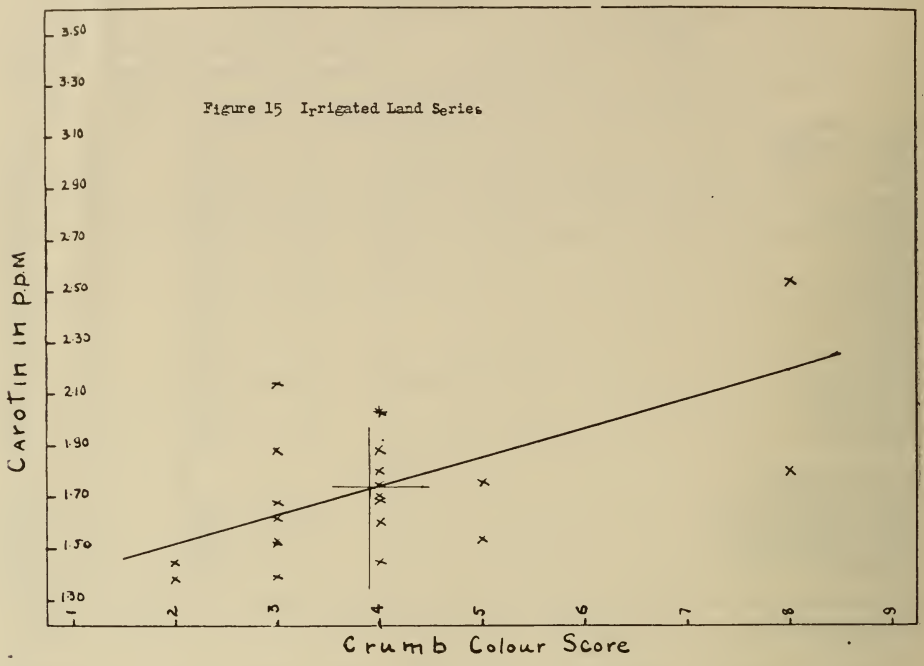
Variety	Dry land series		Irrigated land series	
	Crumb colour	Carotin of flour	Crumb colour	Carotin of flour
	10–1	p.p.m.*	10–1	p.p.m.*
Red Spring Wheats—				
Early Red Fife	3	2·42	3	2·22
Early Triumph	4	2·05	3	1·62
Garnet	4	2·21	3	1·88
Huron	5	2·41	4	1·80
Kitchener	5	2·34	4	2·02
Marquis	2	1·74	3	1·39
Ceres	6	2·53	4	1·69
Reward	2	1·59	2	1·38
Red Fife	3	2·20	4	1·61
Red Bobs 222	3	1·65	4	1·45
Renfrew	4	2·14	4	2·03
Ruby	7	2·02	3	1·53
Supreme	3	2·37	5	1·76
Marquis 10B	6	2·63	2	1·44
Fisher's 1B	9	3·58	8	2·54
Fisher's 2B	4	2·52	3	1·68
928 Q.Q. 2	6	2·80	7	1·80
White Spring Wheats—				
Hard Federation 71	5	2·11	5	1·54
White Federation 4981	4·5	2·56	4	1·75
Hard Federation 31	4	2·04	4	1·71
Hard Federation 4733	4	2·55	4	1·88
Quality	4	1·87	3	1·52

* Parts per million.

The correlation coefficients $r = 0{\cdot}7301 \pm 0{\cdot}0671$ for the dry land series and $r = 0{\cdot}5798 \pm 0{\cdot}0954$ for the irrigated land series were obtained and these



values are illustrated in the correlation surfaces of figures 14 and 15. These data indicate that a fairly close relationship exists between the estimated crumb colour figures for yellowness and the carotin concentrations of the flour.



PART II

The second part of the study was directed toward an investigation of a suitable method for obtaining a quantitative measurement of the pigments in whole wheat which would indicate in a fair degree the yellow colour values which might be expected from the flour milled from it. Such a method would not only save the time required to mill the wheat into flour before determining the yellow pigment values, but would eliminate the necessity of requiring wheat samples in the order of one pound or more to produce the flour. It is conceivable that samples of a few grammes would be sufficient to conduct colour measurements on the whole wheat. The advantages to the plant breeder who is desirous of eliminating lines in the early generations on the basis of carotinoid pigment values without having to multiply those lines to a considerable extent before obtaining a requisite milling sample, can hardly be estimated. Then, too, the mill control laboratory may find use for such a method, especially in the selection of suitable parcels of durum wheat for the production of semolina for the macaroni, vermicelli, etc., trade.

THE EFFECT OF FINENESS OF GRANULATION ON COLOUR DETERMINATIONS

Coleman and Christie (1926) indicated that difficulties arose regarding the preparation of the sample in the gasoline colour test applied to wheat. They concluded that satisfactory results were obtained if the sample was ground so that at least 75 per cent of it passed through a No. 50 grit gauze sieve. Fineness of granulation appeared to be a limiting factor in the amount of colour pigment extracted by gasoline. A prelimary experiment was conducted to determine what effect the preparation of the sample had on the colour deter-

minations and the results reported in Table X corroborated the work of Coleman and Christie that it is necessary to reduce the wheat to a fine degree in order to obtain a reasonable extraction of the pigment. The sample of Ceres wheat, coarsely ground to pass through ½ m.m. sieve, yielded approximately one half as much of the pigments (calculated as carotin) as was obtained from the same sample finely ground on the Seck mill (burr type) where approximately eighty per cent passed through a No. 50 grit gauze. Likewise in the mill run samples of the 1929 and 1930 crops secured from the Minnesota State Testing Mill, the effect of granulation was clearly shown. It will be observed that the values obtained on the finely ground wheat samples approximated the values obtained on the straight grade flours milled from the same wheats.

TABLE X—THE EFFECT OF FINENESS OF GRANULATION ON COLOUR DETERMINATIONS OF GASOLINE EXTRACTS

Sample	Flour colour, carotin*	Wheat colour, ground in Wiley mill (2 m.m. mesh) carotin*	Wheat colour, ground in Wiley mill (½ m.m. mesh) carotin*	Wheat colour, ground in Seck mill (very fine) carotin*
	p.p.m.**	p.p.m.**	p.p.m.**	p.p.m.**
Ceres (U. of Minn. 1929 crop)	.....	.....	0·95	1·88
Mill run (Minn. State T. Mill 1929 crop)	2·21	.....	1·36	2·00
Mill run (Minn. State T. Mill 1930 crop)	2·05	1·10	1·52	2·17

* Values corrected to 13·5 per cent moisture basis.
** Parts per million.

AN EXPERIMENT TO OVERCOME THE EFFECT OF GRANULATION

It will be inferred from the preliminary experiments on the effect of granulation in the extraction of the pigments that it would be desirable if some procedure were found whereby differences in granulation could be overcome by certain manipulations. An attempt was, therefore, made to evolve such a method.

In the determination of fat in bread a method has been suggested by the Bureau of Chemistry, Washington, and referred to in a report of the Connecticut Agricultural Experiment Station, Bulletin 200 (1917), in which a mixture of 10 c.c. ethyl alcohol (95 per cent), 2 c.c. concentrated ammonia and 3 c.c. of water are used. This mixture contributed to a greater increase in the amount of fat which could be extracted from bread than by the use of the official method of extracting fats from food products employed at that time. With this as a lead, many preliminary experiments were conducted to determine if this mixture could be applied to ground wheat in the determination of the carotinoid pigments, as it was thought that a manipulation which would aid in the extraction of fat might have some bearing on the amount of pigment which could be gotten out of ground wheat.

The method finally adopted which seemed to lend itself to satisfactory manipulation is outlined as follows:—

To 20 grammes of the ground wheat contained in a ground glass stoppered reagent bottle of suitable capacity add sufficient of the ammoniacal alcohol mixture (2 c.c. concentrated ammonia, 10 c.c. ethyl alcohol (95%), 3 c.c. distilled water) to wet the meal with no excess of the solution. (This was found to be apparently 15 c.c. for wheat meal ground to pass through a ½ m.m. sieve in a Wiley mill, or 20 c.c. for wheat meal finely ground in the Seck mill referred to elsewhere.) Digest at room temperature for 10 minutes and then introduce 100 c.c. of high grade Cleaner's Naphtha. Shake vigorously by hand, to disintegrate the more or less clumped mass resulting from the action of the ammoniacal alcohol. The bottle is then placed in a shaking machine and shaken for 10 minutes to further insure the penetration of the Naphtha into the

more or less agglutinated particles. It is then set aside in a dark cupboard over night. In the morning the bottle is again shaken vigorously by hand and returned to the shaking machine to be shaken for 20 minutes. The supernatant liquid is poured into a suitable centrifuge; the neck of the tube is covered with rubber dam, and tube and contents are whirled at high speed for 20 minutes. The supernatant liquid is carefully siphoned off by means of a special siphon prepared by drawing out both ends of the glass siphon tube to a capillary, and the end inserted into the liquids is bent back upon itself in the form of a letter "U".

Using the above method as a basis, aliquots of a mill run sample of wheat secured from the Minnesota State mill were submitted to varying methods of manipulation in order to test out the value of the procedure.

These are classified as follows:—

Method I—Preparation of Wheat Sample

Wheat was ground in a Seck mill, a burr type mill, to a very fine meal. This meal fractionated as follows:—

Through No. 10xx silk sieve	43·7 per cent
Over No. 10xx silk sieve	9·0 "
Over No. 72 grit gauze sieve	25·6 "
Over No. 50 grit gauze sieve	21·8 "

Manipulation of Procedure for Extraction of Carotinoid Pigments

(*a*) The meal was extracted with high grade Cleaners Naphtha in the conventional manner outlined for flour on page 9.

(*b*) The meal was extracted according to the method for wheat outlined on page 25 with the exception that the shaking machine was not employed.

(*c*) The method outlined for wheat on page 25 was followed in all details.

(*d*) The method for (C) was followed except that the mixture was shaken for 40 minutes in the shaking machine in the morning.

(*e*) Method for (C) was followed except that mixture was shaken for 60 minutes in the shaking machine next morning.

Method II—Preparation of Wheat Sample

Wheat was ground in a Wiley mill until all of the sample passed through a ½ m.m. screen which is attached to the mill.

Manipulation of Procedure for Extraction of Carotinoid. Pigments

The methods of manipulation for I (*a*), I (*b*), I (*c*), I (*d*), I (*e*) were employed for II (*a*), II (*b*), II (*c*), II (*d*) and II (*e*) respectively.

Method III—Preparation of Wheat Sample

Wheat was ground in a Wiley mill until all of the sample passed through a 2 m.m. screen which is attached to the mill.

Manipulation of Procedure for Extraction of Carotinoid Pigments

(*a*) The same as for I (*a*).

(*c*) The same as for I (*c*).

The data presented in Table XI show a fairly good agreement in the manipulations of the procedure represented in I (*c*), II (*c*), I (*d*), II (*d*), I (*e*) and II (*e*).

TABLE XI—THE EFFECT OF DIFFERENT PROCEDURES IN THE EXTRACTION OF THE PIGMENTS IN WHOLE WHEAT

Designation of method	Transmittancy T	Carotin*	Observations of solution in ultramicroscope
	%	p.p.m.	
I (*a*)	41·0	2·02	Fairly clear of particles.
II (*a*)	49·7	1·58	Fair in clearness.
III (*a*)	60·6	1·14	Fairly clear of particles.
I (*b*)	38·6	2·15	Practically free of particles.
II (*b*)	43·5	1·86	" "
I (*c*)	37·1	2·26	" "
II (*c*)	37·5	2·23	" "
III (*c*)	48·0	1·66	" "
I (*d*)	36·1	2·31	" "
II (*d*)	37·1	2·25	" "
I (*e*)	36·3	2·31	" "
II (*e*)	37·1	2·25	" "

* Transmittancy values were calculated into parts per million of carotin.

These would seem to indicate that wheat ground to pass through ½ m.m. mesh sieve or finer and the meal digested with the ammoniacal alcohol mixture, then extracted with naphtha, gave fairly constant results when the essential details of shaking outlined in the method described on page 25 were adhered to. Shaking in the morning for a longer period than 20 minutes in the shaking machine did not increase the amount of pigments extracted. I (*a*), II (*a*), III (*a*), II (*b*) and III (*c*) gave values considerably lower than the other group. Methods II (*a*), III (*a*), II (*b*) and III (*c*) could not be considered satisfactory in the extraction of the pigments from wheat. The extracts from the ground wheat which were digested with ammoniacal alcohol solution apparently possess a sharper refractive index than those which were not treated, and when viewed in the ultra microscope were practically free of colloidal particles. In addition, the ground meal of the digested mixture flocculated into small particles which soon precipitated to the bottom of the bottle and left the supernatant liquid quite clear. In the undigested mixture the meal did not appear to change and the supernatant liquid was usually quite turbid. It would seem that the starch particles approaching colloidal size were present in the latter mixture but in the digested mixture these appeared to be bound up into the flocculant material.

A STUDY OF THE EXTRACTION OF CAROTINOID PIGMENTS FOR DIFFERENT VARIETIES

From the study of suitable methods for extracting the carotinoid pigments from the wheat it was thought desirable to compare the best of these when applied to a number of wheat varieties. Samples from eight varieties of spring wheat and one of winter wheat were secured from samples grown in $\frac{1}{40}$th acre varietal test plots at the University of Minnesota in 1930. Methods I (*a*), I (*c*) and II (*c*) were applied to the wheats and the conventional method for flour (see page 9) to a 75 per cent patent flour milled from them. The transmittancy readings of the naphtha extracts were calculated to carotin concentrations in parts per million.

Table XII gives a comparison of the results from the different methods used. Figures 16, 17, 18, give a diagrammatic presentation of the results for each method in relation to the carotin concentrations of the flours.

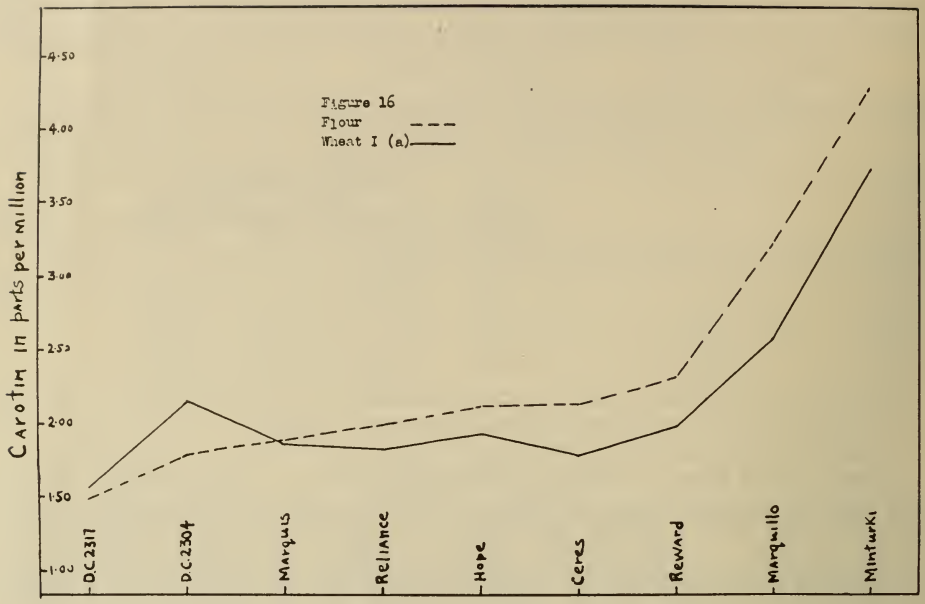


Figure 16
Flour
Wheat I (a)
Carotin in parts per million
4·50
4·00
3·50
3·00
2·50
2·00
1·50
1·00
D.C.2317
D.C.2304
Marquis
Reliance
Hope
Ceres
Reward
Marquillo
Minturki

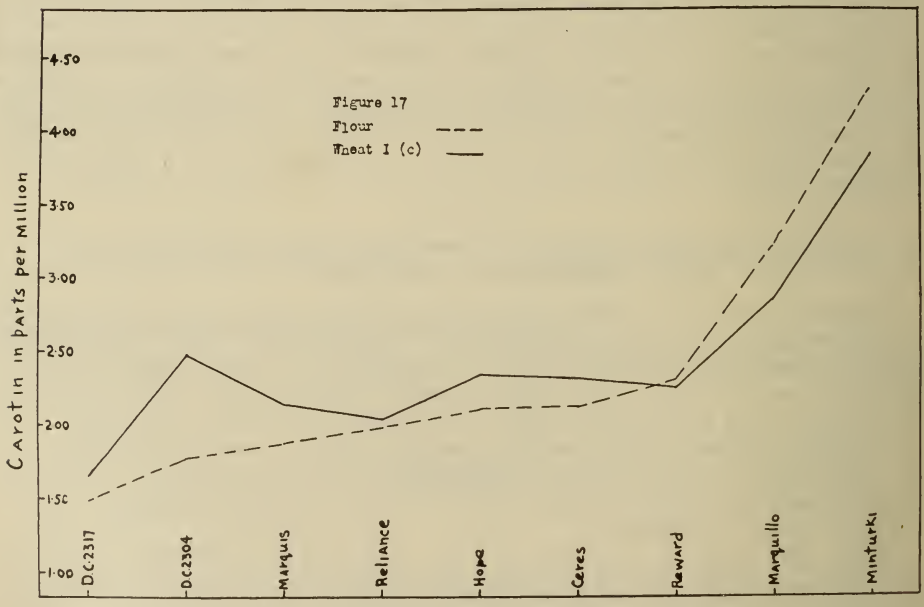


Figure 17
Flour
Wheat I (c)
Carotin in parts per Million
4·50
4·00
3·50
3·00
2·50
2·00
1·50
1·00
DC2317
DC2304
Marquis
Reliance
Hope
Ceres
Reward
Marquillo
Minturki

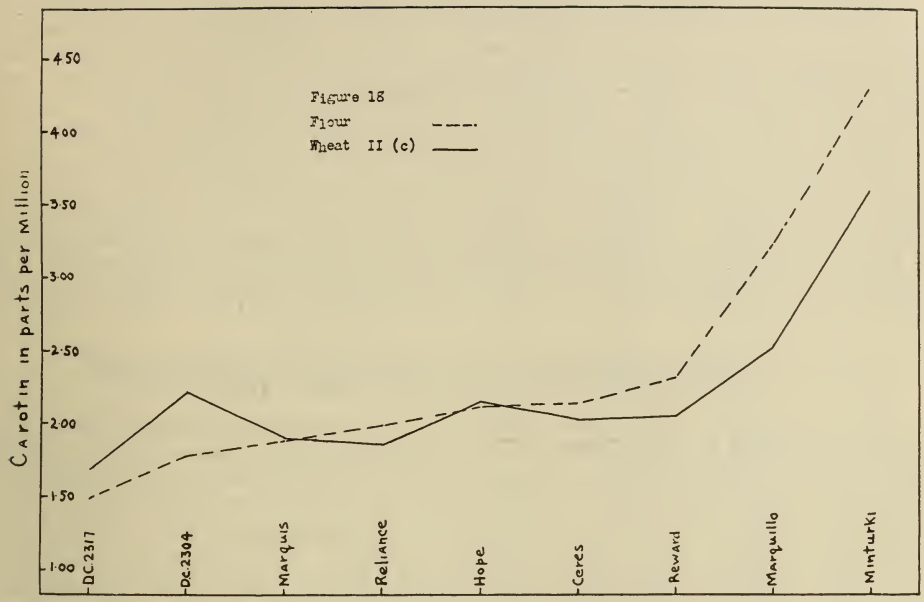


TABLE XII—QUANTITATIVE CAROTINOID EXTRACTION VALUES CALCULATED IN PARTS PER MILLION OF CAROTIN ON NINE VARIETIES OF WHEAT, EMPLOYING DIFFERENT METHODS OF PROCEDURE

—	D. C. 2317	D. C. 2304	Marquis	Reliance	Hope	Ceres	Reward	Marquillo	Minturki
Flour colour.....	1·49	1·78	1·88	1·98	2·11	2·13	2·31	3·23	4·29
Ground wheat I (*a*).....	1·56	2·15	1·85	1·82	1·93	1·77	1·97	2·68	3·73
I (*a*) minus flour colour...	+0·07	+0·37	−0·03	−0·16	−0·18	−0·36	−0·34	−0·55	−0·56
Ground wheat I (*c*).....	1·65	2·48	2·14	2·04	2·34	2·33	2·26	2·86	3·84
I (*c*) minus flour colour...	+0·16	+0·70	+0·26	+0·06	+0·23	+0·20	−0·05	−0·37	−0·45
Ground wheat II (*c*).....	1·69	2·22	1·90	1·86	2·15	2·02	2·05	2·52	3·58
II (*c*) minus flour colour..	+0·20	+0·44	+0·02	−0·12	+0·04	−0·11	−0·26	−0·71	−0·61

Method I (*a*) gave lower values on the wheat than on the flour in seven out of nine wheat samples. These values are considerably lower in the samples with the greater carotin concentrations in the flours. Method I (*c*) gave higher values on the wheat than on the flour in six out of the nine samples. Method II (*c*) gave lower values on the wheat than on the flour in five out of the nine samples. With the exception of the carotin concentration value obtained on D. C. 2304, Method I (*c*) gave results corresponding more closely to those on the flours than either method I (*a*) or II (*c*). Sample D. C. 2304 gave appreciably higher carotin concentration values on the wheat than the carotin concentration on the flour milled from it by all three methods. This may be explained on the assumption that a greater proportion of carotinoid pigments were present in that part of the wheat kernels which did not enter into the patent flour.

Correlation coefficients $r = 0{\cdot}9580 \pm 0{\cdot}0185$, $r = 0{\cdot}9474 \pm 0{\cdot}0023$, and $r = 0{\cdot}9476 \pm 0{\cdot}0029$ were obtained between the carotin concentrations of the flours and the carotin concentration values of the wheats for methods I (*a*) and flour colour, I (*c*) and flour colour, and II (*c*) and flour colour. All these methods gave high significant correlation coefficients between carotin concentrations on the flour and carotin concentration values on the wheat.

In view of the fact that method I (*c*) gave values more closely corresponding to the carotin concentrations of the flours and, in addition, lends itself to a simple manipulation whereby extracts practically free of particles can be obtained, the author suggests, tentatively, that this method is a desirable one for the determination of carotinoid pigments on the whole wheat, which will indicate in a fair degree the carotin concentrations expected from the flour milled from the wheat.

A STUDY OF THE EXTRACTION OF CAROTINOID PIGMENTS FOR DIFFERENT SAMPLES OF THE SAME VARIETY

To investigate further the extraction of carotinoid pigments in relation to wheat varieties fourteen samples each of Marquis and Garnet from the 1930 crop were secured from widely scattered districts in the provinces of Manitoba, Saskatchewan and Alberta. The conventional method for flour (see page 9) except that high grade cleaner's naphtha was substituted for gasoline, was used on the straight grade flours from each sample. A modification of Method I (*a*) which employed the shaking machine as in I (*c*) and which will be designated as I (*g*) and method I (*c*) were followed in obtaining the colour values for the wheat.

The data on the colour values calculated as carotin in parts per million together with the test weight per bushel for each sample are recorded in Table XIII. In an examination of the data it will be observed that considerable variation exists between the values for the flour and wheat colour for each variety. For the straight grade flours the mean value for Marquis is 1·97 and the maximum and minimum are 2·69 and 1·44 parts per million of carotin. In the case of the variety Garnet the mean, maximum and minimum flour colour values are 2·45, 3·86 and 1·79 parts per million of carotin. Similar variations are shown in the wheat colour values. The mean colour values for Marquis and Garnet would indicate that samples of the latter variety may be expected to carry a greater quantity of carotinoid pigments than the former. On the other hand, maximum and minimum colour values for each variety would indicate that certain samples of Garnet may be expected to be lower than certain samples of Marquis in quantity of carotinoid pigments. Colour values determined on the wheat indicate in a fair degree the colour values to be expected in the flour milled from the wheat. Colour values obtained on the wheat by Method I (*g*) were in every case lower than colour values determined by method I (*c*) on eight samples for each variety investigated. On this series of samples the results would appear to indicate that method I (*g*) was more desirable than method I (*c*) when applied to the wheat in indicating the colour values to be expected from the flour milled from the wheat, although it should not be interpreted that method I (*c*) is not a desirable method for determining the carotinoid pigment values on wheat.

Some indication is again in evidence that there is a tendency for a greater quantity of carotinoid pigments to be present in samples of the same variety which are lower in test weight per bushel in comparison with those higher in test weight per bushel. Attention is especially drawn to the samples of Garnet designated as 30·376 and 30·433 which are low in test weight per bushel and high in carotin concentration of flour and of wheat. Correlation coefficients $r = -{\cdot}5120 \pm {\cdot}1455$ and $r = -{\cdot}7583 \pm {\cdot}0766$ were obtained between test weight per bushel and carotin concentration of flour for Marquis and Garnet respectively.

TABLE XIII—COLOUR VALUES AND TEST WEIGHT PER BUSHEL OF DIFFERENT SAMPLES

Mill No.	Variety	Source of sample	Test weight per bushel	Flour colour carotin	Wheat colour	
					Method 1g. carotin	Method 1c. carotin
			lb.	p.p.m.	p.p.m.	p.p.m.
30·359	Marquis	Oakner, Man	61·9	2·30	2·19	.....
30·361	Garnet	"	63·1	2·30	2·46	.....
30·374	Marquis	Katrime, Man	59·0	2·69	2·91	3·42
30·376	Garnet	"	55·8	3·86	3·71	5·19
30·160	Marquis	Indian Head, Sask	63·6	2·28	2·17	.....
30·154	Garnet	"	63·6	2·61	2·76	.....
30·297	Marquis	Rosthern, Sask	64·6	1·83	1·68	.....
30·294	Garnet	"	64·4	2·12	2·40	.....
30·276	Marquis	Scott, Sask	62·8	1·73	1·81	.....
30·272	Garnet	"	63·3	2·12	1·75	.....
30·377	Marquis	Corinne, Sask	61·2	1·63	1·56	1·88
30·379	Garnet	"	60·9	2·13	1·97	2·86
30·413	Marquis	Pathlow, Sask	64·3	2·21	2·65	.....
30·415	Garnet	"	65·5	2·89	3·01	.....
30·422	Marquis	Meota, Sask	64·3	1·87	2·46	3·03
30·424	Garnet	"	64·6	2·32	2·30	2·93
30·431	Marquis	Preeceville, Sask	63·7	2·22	2·36	2·83
30·433	Garnet	"	58·1	3·41	3·00	4·28
30·343	Marquis	Lethbridge, Alta	65·2	1·47	1·68	1·75
30·341	Garnet	"	64·2	1·79	1·87	2·60
30·383	Marquis	Rockfort Bridge, Alta	66·7	1·61	1·63	2·13
30·385	Garnet	"	64·5	2·30	2·18	2·85
30·386	Marquis	Spirit River, Alta	63·8	2·25	2·74	3·09
30·388	Garnet	"	65·3	2·25	2·50	3·20
30·392	Marquis	Fort Sask., Alta	64·2	1·98	2·33	.....
30·394	Garnet	"	65·0	2·27	2·41	.....
30·395	Marquis	Sedgewick, Alta	63·3	1·44	1·82	2·40
30·397	Garnet	"	63·8	1·98	2·27	2·99
Mean and P. E. Marquis			63·5±0·34	1·97±0·07	2·14±0·08	2·57±·11
Maximum Marquis			66·7	2·69	2·91	3·42
Minimum Marquis			59·0	1·44	1·56	1·75
Mean and P.E. Garnet			63·0±0·51	2·45±0·10	2·47±0·09	3·36±0·16
Maximum Garnet			65·5	3·86	3·71	5·19
Minimum Garnet			55·8	1·79	1·75	2·60

SUMMARY

Part I

1. Carotin concentrations were determined spectrophotometrically, on experimentally milled flours from a series of spring wheat varieties grown on dry land and on irrigated land in 1929.
2. Flours milled from dry land wheat were higher in carotin concentrations than flours milled from irrigated land wheat.
3. An inherent varietal influence contributed to the carotin concentrations obtained in the dry land samples in comparison with the carotin concentrations obtained in the irrigated land samples.
4. Test weight per bushel was negatively correlated with carotin concentration of flour.
5. Weight per 1,000 kernels was negatively correlated with carotin concentration of flour.
6. Protein content of wheat was not considered to be correlated with carotin concentration of flour.
7. Ash percentage of flour was not considered to be correlated with carotin concentration of flour.
8. Dry and wet slick colour estimations did not appear to be reliable in estimating carotin concentrations of flour.
9. Crumb colour comparisons were significantly correlated with carotin concentrations of flour.

Part II

10. A suitable method for the determination of the carotin concentration values of the wheat which would indicate in a fair degree, the carotin concentration to be expected from the flour milled from it is, tentatively, suggested.
11. Samples of the same variety may be expected to vary considerably in carotin concentration of the wheat and of the flour milled from the wheat. Higher values appear to be associated with lack of plumpness as determined by test weight per bushel measurements.

BIBLIOGRAPHY

1. Coleman, D. A. and Christie, Alfred
1926. The gasoline colour value of several classes of wheat. Cereal Chemistry, 3: 188
2. Capper, N. S.
1930. The transformation of carotene into vitamine A, as shown by a study of the absorption spectra of rat liver oils.
Biochem, J. XXIV: 980
3. Ferrari, C. G. and Bailey, C. H.
1930. Carotinoid pigments of flour.
Cereal Chemistry, 6: 218
4. Ferrari, C. G. and Bailey, C. H.
1930. The determination of carotin in flour.
Cereal Chemistry, 6: 347
5. Geddes, W. F.
1931. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures, I.
Can. Jour. of Research 1: 528–556.
6. Gullard, J. M.
1930. The chemical constitution of the carotinoid pigments and the relation of carotene to vitamine A.
Chem. and Ind. XLIX: 839-847

7. Jørgensen, Holger
1927. Use of chromate solutions as comparison-standards for the determination of gasoline colour standards.
Cereal Chemistry, 4: 468

8. Kent-Jones, D. W. and Herd, C. W.
1927. A numerical expression for the colour of flour.
Analyst, 52: 443-452

9. Monier-Williams, G. W.
1912. Report to the local government board on the nature of the colouring matter of flour and its relation to the processes of natural and artificial bleaching.
Repts. Local Govt. Bd. (Great Brit.) Pub. Health and Med. Subjs., n-ser., No. 73

10. Moore, T.
1930. V. The absence of the liver oil vitamine A from carotene
VI. The conversion of carotene to vitamine A in vivo.
Biochem. J. XXIV: 692, 702

11. Palmer, L. S.
1922. Carotinoids and related pigments.
Chem. Cat. Co., New York.

12. Schertz, F. M.
1923. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter.
J. Agr. Research, 26: 383-400

13. Schertz, F. M.
1925. Some physical and chemical properties of carotin and the preparation of the pure pigment.
J. Agr. Research, 30: 469-474

14. Spalding, J. L.
1930. Quick ash determination by magnesium acetate-alcohol method.
Cereal Chemistry, 7: 93-97

15. Visser't Hooft, F., and deLeeuw, F. J. G.
1928. A critical study of some methods used in flour colorimetry.
Cereal Chemistry, 5: 351-365

16. Willstätter, R. and Stoll, A.
1913. Untersuchungen über Chlorophyll Methoden und Ergebnisse.
Berlin.

17. Winton, A. L.
1911. Colour of flour and a method for the determination of gasoline colour value.
U.S. Dept. Agr. Bur. Chem. Bull. 137: 144-148.

GENERAL REFERENCES

1. Bailey, C. H.
1925. The chemistry of wheat flour.
Chem. Cat. Co., New York.

2. Gortner, R. A.
1929. Outlines of biochemistry.
John Wiley and Sons, New York.

3. Hayes, H. K. and Garber, R. J.
1927. Breeding crop plants.
McGraw Hill Book Co., New York.

4. Schertz, F. M. and Merz, A. R.
1928. English translation of "Investigations on Chlorophyll" by Richard Willstätter and Arthur Stoll.
The Science Press Printing Co., Lancaster, Pa.

5. Spoehr, H. A.
1926. Photosynthesis.
Chem. Cat. Co., New York.

ACKNOWLEDGMENT

The foregoing studies were largely made possible through the opportunities afforded the author when enrolled as a graduate student at the University of Minnesota, U.S.A. The author is deeply indebted to Dr. C. H. Bailey, Professor of Agricultural Biochemistry and his Co-workers for guidance and helpful suggestions in outlining and conducting these studies and to the University of Minnesota for providing necessary facilities.

CEREAL DIVISION

L. H. Newman, B.S.A., Dominion Cerealist

Cerealists.

J. G. C. Fraser, B.S.A., Chief Assistant—
Spring Wheat Breeding and Investigations.

P. R. Cowan, B.S.A.—
Barley and Pea Breeding and Investigations.

A. G. O. Whiteside, B.S.A., M.S.—
Flour Quality Investigations.
Autumn Wheat and Rye Breeding and Investigations.

R. A. Derick, B.S.A., M.Sc.—
Oat Breeding and Investigations.
Grain Mixtures.

W. G. McGregor, B.S.A., M.S.—
Flax (for seed), Hemp, Buckwheat and Bean Breeding and Investigations.

Rust Laboratory, Winnipeg, Man.:

Senior Cereal Specialist.....C. H. Goulden, Ph.D.
Cerealist Specialists.....K. W. Neatby, M.S.A., Ph.D.
E. Lillian Hollingshead, M.A., Ph.D.
Cerealist.....J. N. Welsh, M.S.A.

OTTAWA
F. A. ACLAND
PRINTER TO THE KING'S MOST EXCELLENT MAJESTY
1931